

PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (<http://bmjopen.bmj.com/site/about/resources/checklist.pdf>) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Effects of short-term exposure to air pollution on hospital admissions for autism spectrum disorder in Korean school-aged children: A nationwide time-series study
AUTHORS	Kim, Kyoung-Nam; Sohn, Ji Hoon; Cho, Sung Joon; Seo, Hwo Yeon; Kim, Soontae; Hong, Yun-Chul

VERSION 1 – REVIEW

REVIEWER	BAGHDADLI, AMARIA CHU of Montpellier, Cente de ressources autisme
REVIEW RETURNED	31-Jan-2022

GENERAL COMMENTS	<p>Ref: BMJ open-2021-058286</p> <p>Title: Effects of short-term exposure to air pollution on hospital admissions for autism spectrum disorder in school-aged children: a nationwide time-series study</p> <p>I was very interested in reading this interesting and very original article. The aim of this ecological study was to determine whether short-term exposure to air pollution was associated with hospital admissions, a proxy of symptom aggravation, in children with autism spectrum disorder (ASD) in Korea.</p> <p>The effects of short-term exposure were explored for PM2.5, NO2 and O3 concentrations at the regional level, for up to 6 days before hospital admissions using time series analyses over the 2011-2015 period. Data about hospital admissions were derived from the National Health Insurance Service.</p> <p>The results showed that Short-term exposures to PM2.5, NO2, and O3 were associated with a higher risk of hospital admissions for ASD, with different lag period for each pollutant.</p> <p>General comments</p> <p>This is a well-written paper reporting interesting results as there have been very few studies attempting to determine whether environmental factors may influence the severity of the clinical manifestations in children with ASD.</p> <p>However, there are several aspects of the study that require more details and discussion. In particular, a more comprehensive description of the National Health Insurance, the hospital admissions data and the pollutants concentrations at the regional level would allow the readers to gain deeper insights about the significance of the results.</p> <p>I. Major comments</p>
-------------------------	---

	<p>1. The authors conducted a nation-wide study, using the 2010 census to collect data about population at the regional level. In order to appreciate the possibility of a selection bias in the ascertainment of hospital admissions, it would be truly informative to precise i) the percentage of Korean people who are beneficiary of the National Health Insurance (probably close to 100%?) at the country level, and ii) to precise whether this percentage is similar between the 16 regions.</p> <p>2. ASD symptom aggravation frequency is evaluated using the number of hospital admission per day and by region. Is hospital settings the only place, which take care of ASD children with aggravated symptoms in Korea or private practice is also an option? It needs to be specified. In the case where private practice is likely, the possibility of an underestimation of the number of aggravation cases should be discussed. Furthermore, it could be assumed that the access to hospital facilities is more difficult in the most rural regions, which are also the least exposed, leading as well in such cases to an underestimation of the cases. Did the authors consider to carry out sensitivity analyses stratified on the regions' characteristics?</p> <p>3. The authors wrote in the discussion section that "predetermined scheduled hospital admissions can also be another source of outcome misclassification". It should be specified as soon as the methods section whether the authors considered any admission whatever its form (i.e., hospitalization, emergency visit, follow-up visit). If data provided by the national health insurance inform about the nature of admission, it should be described and analyses carried out excluding follow-up visits that do not involve systematically an aggravation of the symptoms.</p> <p>4. Exposure to air pollution was estimated at the regional level and consequently, the daily average pollutants concentrations are given for very large areas. Within these areas, the heterogeneity in pollutants levels may be high, especially for NO₂, leading to exposure misclassification. Did the authors consider to estimate pollutants concentrations in the vicinity of the hospitals or at least in census areas of smaller size than the regional one? It could limit the risk of such a bias. At least, this point needs to be discussed.</p> <p>5. The association with PM_{2.5} is significant for PM_{2.5} levels at lag day 1, which represents a very short delay between the exposure and the outcome. Do the authors think that it could be the consequence of strong and very short-term effects of PM_{2.5} that motivate hospital admission in emergency? It is all the more surprising because exposure to lag day 0 shows the opposite effect. The authors explain this observation by a potential harvesting effect. Could they provide a graph showing the daily distribution of pollutants and daily hospital admission at least on a short period to illustrate this hypothesis?</p> <p>II. Minor comments</p> <p>8. A reference is required P7L49 to support the statement that short-term exposure to air pollution can induce neuroinflammation and systemic inflammation.</p>
--	---

REVIEWER	Bernardini, Francesco Azienda Sanitaria Friuli Occidentale
REVIEW RETURNED	09-Feb-2022

GENERAL COMMENTS	<p>This is a time-series analysis on the association of 3 air pollutants levels and daily hospital admissions for autism spectrum disorder in the Republic of Korea.</p> <p>The topic of air pollution as risk factor for mental health, including autism spectrum disorder, is important. The article is well written, informative and well-referenced.</p> <p>As a reviewer I have the following remarks:</p> <ol style="list-style-type: none"> 1) In their introduction the authors introduced the concept of air pollution as a potential mediator of neuroinflammation and systemic inflammation without referencing. Please further discuss this very important point. 2) Inflammatory processes are not the only potential mechanisms potentially implicated in the influence of air pollution on the central nervous system. A short but informative paragraph on the biological hypothesis of how air pollution could have an effect on the brain and central nervous system would be greatly appreciated (please see: Block and Calderón-Garcidueñas. Air pollution: Mechanisms of neuroinflammation and CNS disease. Trends Neurosci, 2009) 3) There is increasingly emerging literature analyzing the link between air pollution levels and poor mental health. In particular, as the present study focus on the short-term effect of air pollution exposure on ASD symptom aggravation, as measured by daily hospital admissions, I suggest to provide a short background paragraph about short-term air pollution exposure and hospital admissions for psychiatric disorders in general (please see: Bernardini et al. Air Pollutants and Daily Hospital Admissions for Psychiatric Care: A Review. Psychiatric Services, 2020)
-------------------------	---

VERSION 1 – AUTHOR RESPONSE

Reviewer #1

I was very interested in reading this interesting and very original article. The aim of this ecological study was to determine whether short-term exposure to air pollution was associated with hospital admissions, a proxy of symptom aggravation, in children with autism spectrum disorder (ASD) in Korea.

The effects of short-term exposure were explored for PM2.5, NO2 and O3 concentrations at the regional level, for up to 6 days before hospital admissions using time series analyses over the 2011–2015 period. Data about hospital admissions were derived from the National Health Insurance Service.

The results showed that short-term exposures to PM2.5, NO2, and O3 were associated with a higher risk of hospital admissions for ASD, with different lag period for each pollutant.

General comments

This is a well-written paper reporting interesting results as there have been very few studies attempting to determine whether environmental factors may influence the severity of the clinical manifestations in children with ASD.

However, there are several aspects of the study that require more details and discussion. In particular, a more comprehensive description of the National Health Insurance, the hospital admissions data and the pollutants concentrations at the regional level would allow the readers to gain deeper insights about the significance of the results.

Major comments

1. The authors conducted a nation-wide study, using the 2010 census to collect data about population at the regional level. In order to appreciate the possibility of a selection bias in the ascertainment of hospital admissions, it would be truly informative to precise i) the percentage of Korean people who are beneficiary of the National Health Insurance (probably close to 100%?) at the country level, and ii) to precise whether this percentage is similar between the 16 regions.

Response: Thank you for your valuable comments. All the residents of the Republic of Korea can access health care regardless of their ability to pay, through the National Health Insurance, which covers 97% of the population, and the Medical Aid program, which covers 3% of the population. The data provided by the National Health Insurance Service included information on the population covered by the National Health Insurance and the population covered by the Medical Aid program. Therefore, it virtually had information on all residents of the Republic of Korea, and selection bias according to the region was not expected. We agree that this information was not sufficiently described in the original manuscript. Therefore, we have revised the manuscript as follows:

Original:

“The data were aggregated according to the 16 regions of the Republic of Korea (Online Supplementary Table 1) and sex and provided by the National Health Insurance Service, the sole health insurance provider of the universal coverage system.”

Revised:

“The data were aggregated according to the 16 regions of the Republic of Korea (Online Supplementary Table 1) and sex and provided by the National Health Insurance Service, the sole health insurance provider of the universal coverage system. The National Health Insurance Service had medical information on all the residents of the Republic of Korea, including the data of those covered by either the National Health Insurance (97%) or the Medical Aid program (3%) [14].”

14 Yu TY, Cho H, Kim TY, et al. Utilization of Osteoporosis-Related Health Services: Use of Data from the Korean National Health Insurance Database 2008-2012. *J Korean Med Sci* 2018;**33**:e20. doi:10.3346/jkms.2018.33.e20

Page 7 (METHODS)

2. ASD symptom aggravation frequency is evaluated using the number of hospital admission per day and by region. Is hospital settings the only place, which take care of ASD children with aggravated symptoms in Korea or private practice is also an option? It needs to be specified. In the case where private practice is likely, the possibility of an underestimation of the number of aggravation cases should be discussed. Furthermore, it could be assumed that the access to hospital facilities is more difficult in the most rural regions, which are also the least exposed, leading as well in such cases to an underestimation of the cases. Did the authors consider to carry out sensitivity analyses stratified on the regions' characteristics?

Response: Thank you for your insightful comments, which have helped us improve the quality of the study. As you indicated, private practice or community-based programs are also potential options for taking care of ASD children with aggravated symptoms in Korea. We agree that this could have led to an underestimation of the effects of air pollution on ASD symptom aggravation reported in the present study.

When we further conducted stratified analyses by region characteristics [seven metropolitan cities (Seoul, Busan, Daegu, Incheon, Gwangju, Daejeon, and Ulsan) vs. nine non-metropolitan regions (Gyeonggi-do, Gangwon-do, Chungcheongbuk-do, Chungcheongnam-do, Jeollabuk-do, Jeollanam-do, Gyeongsangbuk-do, Gyeongsangnam-do, and Jeju-do)] (Online Supplementary Table 1) according to your suggestion, we found that the associations between air pollution exposures and hospital admissions for ASD were generally more prominent in the nine non-metropolitan regions than that in the seven metropolitan cities, although the CIs overlapped (Online Supplementary Table 2).

The reason for this finding is unclear. However, we assume that the more prominent associations in the non-metropolitan regions than in metropolitan cities might be explained at least in part by the fact that most resources for these treatment options other than those in the hospitals (private practice and community-based programs), were in the metropolitan cities. Because Korea has universal health coverage and access to hospitals is very high, in regions other than the metropolitan cities, people might rely more on the hospitals when ASD symptoms are aggravated.

We have revised the manuscript as follows:

Original:

“We also performed stratified analyses according to sex.”

Revised:

“We also performed stratified analyses according to sex and region characteristics [seven metropolitan cities (Seoul, Busan, Daegu, Incheon, Gwangju, Daejeon, and Ulsan) vs. nine non-metropolitan regions (Gyeonggi-do, Gangwon-do, Chungcheongbuk-do, Chungcheongnam-do, Jeollabuk-do, Jeollanam-do, Gyeongsangbuk-do, Gyeongsangnam-do,

and Jeju-do] (Online Supplementary Table 1). We conducted the regional stratified analyses assuming that hospital visits due to ASD symptom aggravation might differ by region characteristics.”

Page 11 (METHODS)

Original:

“We found consistent associations between PM_{2.5}, NO₂, and O₃ levels and hospital admissions for ASD in the multiple-pollutant model (Online Supplementary Table 2) compared to the main analytical models.”

Revised:

“In the analyses stratified by region characteristics, the associations between air pollution exposures and hospital admissions for ASD were generally more prominent in the nine non-metropolitan regions than those in the seven metropolitan cities, although the CIs overlapped (Online Supplementary Table 2).

We found consistent associations between PM_{2.5}, NO₂, and O₃ levels and hospital admissions for ASD in the multiple-pollutant model (Online Supplementary Table 3) compared to the main analytical models.”

Page 18 (RESULTS)

Online Supplementary Table 2. Associations between PM_{2.5}, NO₂, and O₃ levels and hospital admissions for autism spectrum disorder in seven metropolitan cities and nine non-metropolitan regions^a

Seven metropolitan cities			Nine non-metropolitan regions		
PM _{2.5}	NO ₂	O ₃	PM _{2.5}	NO ₂	O ₃

	RR (95% CI)					
Lag day 0	0.91 (0.78, 1.05)	0.75 (0.55, 1.01)	1.00 (0.87, 1.14)	0.79 (0.71, 0.87)*	0.88 (0.69, 1.13)	0.98 (0.89, 1.07)
Lag day 1	1.16 (1.01, 1.34)*	1.07 (0.80, 1.43)	1.11 (0.97, 1.26)	1.19 (1.08, 1.31)*	1.13 (0.89, 1.43)	1.01 (0.92, 1.10)
Lag day 2	0.98 (0.89, 1.07)	0.81 (0.68, 0.97)*	0.97 (0.89, 1.06)	1.03 (0.96, 1.09)	1.23 (1.06, 1.42)*	0.92 (0.87, 0.98)*
Lag day 3	0.91 (0.83, 1.003)	0.83 (0.69, 0.99)*	0.92 (0.84, 1.00)*	0.95 (0.89, 1.02)	1.22 (1.05, 1.41)*	0.97 (0.91, 1.03)
Lag day 4	0.96 (0.89, 1.04)	1.03 (0.90, 1.19)	0.94 (0.87, 1.01)	1.00 (0.94, 1.05)	1.16 (1.03, 1.31)*	1.08 (1.03, 1.14)
Lag day 5	0.99 (0.90, 1.08)	1.16 (0.98, 1.37)	0.97 (0.89, 1.05)	1.03 (0.96, 1.09)	1.09 (0.95, 1.25)	1.11 (1.05, 1.17)*
Lag day 6	0.96 (0.89, 1.03)	1.08 (0.96, 1.22)	0.98 (0.92, 1.05)	1.01 (0.96, 1.06)	1.03 (0.93, 1.14)	1.01 (0.97, 1.05)

^aThe results are presented for a 10.0 µg/m³ increase for PM_{2.5} and 10.0 ppb for NO₂ and O₃, respectively in models adjusted for region, day, temperature, relative humidity, and population. RR = relative risk; CI = confidence interval.

*p <0.05.

Original:

“Sixth, although hospital admissions are commonly used as a proxy for symptom aggravation in epidemiological studies,[11] hospital admissions for ASD might disproportionately reflect aggravation of symptoms related to hyperactivity, aggression, and self-injurious behaviour more than those related to deficits in social communication.”

Revised:

“Sixth, although hospital admissions are commonly used as a proxy for symptom aggravation in epidemiological studies,[11] hospital admissions for ASD might disproportionately reflect aggravation of symptoms related to hyperactivity, aggression, and self-injurious behaviour more than those related to deficits in social communication. In addition, private practice or community-based programs are also potential options for providing care for ASD children with aggravated symptoms in Korea. This could have led to the underestimation of the effect of air pollution on ASD symptom aggravation observed in the present study. We assume that the more prominent associations in the non-metropolitan regions than in metropolitan cities might also be explained at least in part by the fact that most resources for these treatment options other than those in hospitals, were in metropolitan cities.”

Page 23 (DISCUSSION)

- 3. The authors wrote in the discussion section that “predetermined scheduled hospital admission can also be another source of outcome misclassification.” It should be specified as soon as the methods section whether the authors considered any admission whatever its form (i.e., hospitalization, emergency visit, follow-up visit). If data provided**

by the national health insurance inform about the nature of admission, it should be described and analyses carried out excluding follow-up visits that do not involve systematically an aggravation of the symptoms.

Response: Thank you for your valuable comments, which have helped us improve clarity in the manuscript. The National Health Insurance Service provides information on any admission regardless of the route of admission. We agree that this information should be described in the Methods section but was absent in the original manuscript. Therefore, we have added this information in the Methods section as follows:

Original:

“We conducted a time-series study using data on daily counts of hospital admissions for ASD among children aged 5–14 years between January 1, 2011, and December 31, 2015. The data were aggregated according to the 16 regions of the Republic of Korea (Online Supplementary Table 1) and sex and provided by the National Health Insurance Service, the sole health insurance provider of the universal coverage system.”

Revised:

“We conducted a time-series study using data on daily counts of hospital admissions for ASD among children aged 5–14 years between January 1, 2011, and December 31, 2015. The data, which were provided by the National Health Insurance Service, the sole health insurance provider of the universal coverage system, were aggregated according to the 16 regions of the Republic of Korea (Online Supplementary Table 1) and sex. ~ Because the National Health Insurance Service data used in the present study did not disaggregate according to emergency visit or follow-up visit, we considered hospital admissions regardless of the route of admission (e.g., emergency visit, follow-up visit) and could not perform analyses excluding follow-up visits.”

Page 8 (METHODS)

4. **Exposure to air pollution was estimated at the regional level and consequently, the daily average pollutants concentrations are given for very large areas. Within these areas, the heterogeneity in pollutants levels may be high, especially for NO₂, leading to exposure misclassification. Did the authors consider to estimate pollutants concentrations in the vicinity of the hospitals or at least in census areas of smaller size than the regional one? It could limit the risk of such a bias. At least, this point needs to be discussed**

Response: Thank you for your valuable comments. Because the National Health Insurance Service did not provide daily counts of data for spatial units finer than the regional level due to the concern of individual identification issues, we could not conduct analyses for pollutants considering the vicinity of the hospitals or at least in smaller size census areas than the region. Although there have been several time-series studies conducted in Korea using regional-level air pollution concentrations as exposures,[1–3] we agree that exposure misclassification attributable to excessively large spatial units should be discussed more extensively in the Discussion section.

1 Kim K-N, Lim Y-H, Bae S, *et al.* Age-specific effects of ozone on pneumonia in Korean children and adolescents: a nationwide time-series study. *Epidemiol Health* 2022;**44**:e2022002. doi:10.4178/epih.e2022002

2 Park J, Sohn JH, Cho SJ, *et al.* Association between short-term air pollution exposure and attention-deficit/hyperactivity disorder-related hospital admissions among adolescents: A nationwide time-series study. *Environ Pollut* 2020;**266**:115369. doi:10.1016/j.envpol.2020.115369

3 Lee W, Prifti K, Kim H, *et al.* Short-term Exposure to Air Pollution and Attributable Risk of Kidney Diseases: A Nationwide Time-series Study. *Epidemiology* 2022;**33**:17–24. doi:10.1097/EDE.0000000000001430

Therefore, we have revised the manuscript as follows:

Original:

“Fourth, the Berkson measurement error in the evaluation of air pollution exposures is a concern in this time-series study assigning regional air pollution levels as exposures (instead of directly measuring individual exposures). This type of error is likely to lead to imprecision in the estimation of associations rather than bias.”

Revised:

“Fourth, there is a concern of exposure misclassification attributable to large spatial units, although several time-series studies conducted in Korea used regional air pollution levels as exposures (instead of air pollution levels measured for a finer spatial resolution).[41–43] This type of measurement error (i.e., Berkson error) is likely to lead to imprecision in the estimation of associations rather than bias.”

Page 21 (DISCUSSION)

- 5. The association with PM_{2.5} is significant for PM_{2.5} levels at lag day 1, which represent s a very short delay between the exposure and the outcome. Do the authors think that it could be the consequence of strong and very short-term effects of PM_{2.5} that motivate hospital admission in emergency? It is all the more surprising because exposure to lag day 0 shows the opposite effect. Could they provide a graph showing the daily distribution of pollutants and daily hospital admission at least on a short period to illustrate this hypothesis?**

Response: Thank you for your valuable comments, which have helped us improve the quality of the study. According to your suggestion, we have created a plot depicting the daily distributions of pollutants and daily hospital admissions for a short period, to illustrate the short-term effects of PM_{2.5} and a harvesting effect. In this plot showing the daily concentrations of PM_{2.5} and counts of hospital admissions for autism spectrum disorder in Seoul, in January 2015, we found clear paralleled and

lagged (for just a few days) distribution patterns of PM_{2.5} levels and ASD, which, we believe, provides additional support for short-term effects of PM_{2.5} and a harvesting effect (Online Supplementary Figure 3). We have revised the manuscript as follows:

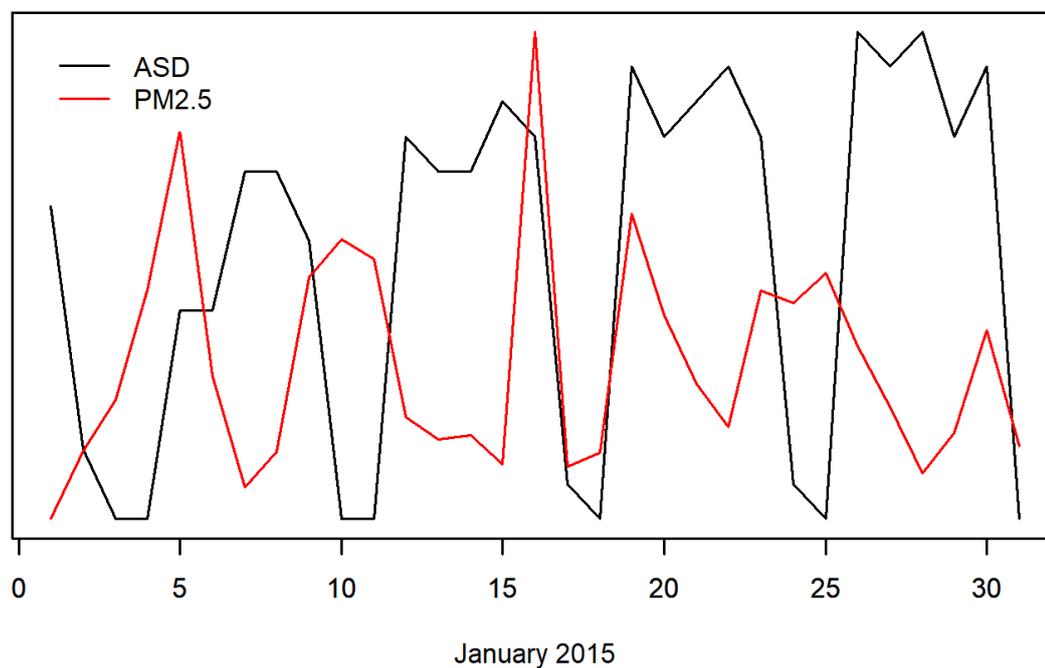
Original:

“Because these inverse associations are clinically irrelevant (given that we considered hospital admissions as the outcome) and biologically implausible (considering the causal pathway via microglia activation and inflammatory status mentioned above), we assumed that the inverse associations between PM_{2.5} and NO₂ levels at lag day 0 and hospital admissions for ASD may be explained by the harvesting effect (outcome displacement) induced by air pollution exposures at previous lag days (e.g., PM_{2.5} at lag day 1 and NO₂ levels at lag day 5).[32]”

Revised:

“Because these inverse associations are clinically irrelevant (given that we considered hospital admissions as the outcome) and biologically implausible (considering the causal pathway via microglia activation and inflammatory status mentioned above), we assumed that the inverse associations between PM_{2.5} and NO₂ levels at lag day 0 and hospital admissions for ASD may be explained by the harvesting effect (outcome displacement) induced by air pollution exposures at previous lag days (e.g., PM_{2.5} at lag day 1 and NO₂ levels at lag day 5).[32] The findings of this study are further supported by the clear paralleled and lagged distribution patterns of the daily PM_{2.5} levels and counts of hospital admissions for ASD (Online Supplementary Figure 3).”

Page 21 (DISCUSSION)



Online Supplementary Figure 3. Daily concentrations of PM_{2.5} and counts of hospital admissions for autism spectrum disorder in Seoul, in January 2015. ASD, autism spectrum disorder.

Minor comment

- A reference is required P7L49 to support the statement that short-term exposure to air pollution can induce neuroinflammation and systemic inflammation.**

Response: Thank you for your helpful comment. Accordingly, we have added the following reference to support the statement as indicated.

Original:

“Short-term exposure to air pollution (i.e., days to weeks) can induce neuroinflammation and systemic inflammation and may aggravate ASD symptoms.”

Revised:

“Short-term exposure to air pollution (i.e., days to weeks) can induce systemic inflammation and neuroinflammation (possibly due to the penetration of particulate air pollutants through the lungs and olfactory epithelium, changes in blood brain barrier, and activation of microglia) and may aggravate ASD symptoms.[10–12]”

10 Li W, Dorans KS, Wilker EH, *et al.* Short-Term Exposure to Ambient Air Pollution and Biomarkers of Systemic Inflammation: The Framingham Heart Study. *Arterioscler Thromb Vasc Biol* 2017;**37**:1793–800. doi:10.1161/ATVBAHA.117.309799

11 Block ML, Calderón-Garcidueñas L. Air pollution: mechanisms of neuroinflammation and CNS disease. *Trends Neurosci* 2009;**32**:506–16. doi:10.1016/j.tins.2009.05.009

12 Campolim CM, Weissmann L, Ferreira CK de O, *et al.* Short-term exposure to air pollution (PM2.5) induces hypothalamic inflammation, and long-term leads to leptin resistance and obesity via Tlr4/Ikbke in mice. *Sci Rep* 2020;**10**:10160. doi:10.1038/s41598-020-67040-3

Page 7 (INTRODUCTION)

Reviewer #2

This is a time-series analysis on the association of 3 air pollutants levels and daily hospital admissions for autism spectrum disorder in the Republic of Korea. The topic of air pollution as risk factor for mental health, including autism spectrum disorder, is important. The article is well written, informative and well-referenced.

As a reviewer I have the following remarks:

1. In the introduction the authors introduced the concept of air pollution as a potential mediator of neuroinflammation and systemic inflammation without referencing. Please further discuss this very important point.

Response: Thank you for your valuable comment, which has helped to improve the quality of the study. As pointed out, the original manuscript required necessary references to substantiate a statement; therefore, we have referenced the statement adequately. In addition, according to your helpful suggestion, we have further discussed this point as follows:

Original:

“Short-term exposure to air pollution (i.e., days to weeks) can induce neuroinflammation and systemic inflammation and may aggravate ASD symptoms.”

Revised:

“Short-term exposure to air pollution (i.e., days to weeks) can induce systemic inflammation and neuroinflammation (possibly due to the penetration of particulate air pollutants through the lungs and olfactory epithelium, changes in blood brain barrier, and activation of microglia) and may aggravate ASD symptoms.[10–12]”

10 Li W, Dorans KS, Wilker EH, *et al.* Short-Term Exposure to Ambient Air Pollution and Biomarkers of Systemic Inflammation: The Framingham Heart Study. *Arterioscler Thromb Vasc Biol* 2017;**37**:1793–800. doi:10.1161/ATVBAHA.117.309799

11 Block ML, Calderón-Garcidueñas L. Air pollution: mechanisms of neuroinflammation and CNS disease. *Trends Neurosci* 2009;**32**:506–16. doi:10.1016/j.tins.2009.05.009

12 Campolim CM, Weissmann L, Ferreira CK de O, *et al.* Short-term exposure to air pollution (PM2.5) induces hypothalamic inflammation, and long-term leads to leptin resistance and obesity via Tlr4/Ikbke in mice. *Sci Rep* 2020;**10**:10160. doi:10.1038/s41598-020-67040-3

Page 7 (INTRODUCTION)

- 2. Inflammatory processes are not the only potential mechanisms potentially implicated in the influence of air pollution on the central nervous system. A short but informative paragraph on the biological hypothesis of how air pollution could have an effect on the brain and central nervous system would be greatly appreciated (please see: Block and Calderon-Garciduenas. Air pollution: Mechanisms of neuroinflammation and CNS disease. Trends Neurosci, 2009)**

Response: Thank you for your valuable comment, which has helped to improve the quality of the study. We agree that a short but informative description of various mechanisms other than inflammatory processes would be helpful for readers to understand the mechanism of the results of this study in a broader context. Therefore, according to your helpful comment, we have added a paragraph describing the mechanisms other than inflammatory processes and cited the reference you suggested, as follows:

Original:

“However, short-term exposure to air pollution is known to activate microglia, the resident immune cells of the central nervous system involving the production and secretion of pro-inflammatory cytokines, such as interleukin (IL)-6, IL-1 β , and tumour necrosis factor (TNF)- α . [12]”

Revised:

“Air pollution is known to impact the central nervous system by activating microglia and disrupting the blood-brain barrier through systemic inflammation, neuroinflammation, oxidative stress, cerebrovascular injury, and neurodegenerative processes. [11] Short-term exposure to air pollution is known to activate microglia, the resident immune cells of the central nervous system involved in the production and secretion of pro-inflammatory cytokines, such as interleukin (IL)-6, IL-1 β , and tumour necrosis factor (TNF)- α . [12]”

11 Block ML, Calderón-Garcidueñas L. Air pollution: mechanisms of neuroinflammation and CNS disease. *Trends Neurosci* 2009;**32**:506–16. doi:10.1016/j.tins.2009.05.009

Page 19 (DISCUSSION)

- 3. There is increasingly emerging literature analyzing the link between air pollution levels and poor mental health. In particular, as the present study focus on the short-term effect of air pollution exposure on ASD symptom aggravation, as measured by daily hospital admission, I suggest to provide a short background paragraph about short-term air pollution exposure and hospital admissions for psychiatric disorders in general (please see: Bernardini et al. Air pollutants and Daily Hospital Admissions for Psychiatric Care: A Review. *Psychiatric Services*, 2020)**

Response: Thank you for your valuable comment. As you indicated, there is increasingly emerging literature demonstrating the link between air pollution levels and poor mental health, which can help the readers to understand the results of this study in a broader context. We found the literature you

indicated to be adequate and very helpful for summarising the above-mentioned subject. Therefore, we have added a short background paragraph and cited the study you suggested as follows:

Original:

“To the best of our knowledge, no direct evidence, except the present study, exists on the association between short-term exposure to air pollution and ASD symptom aggravation.”

Revised:

“Short-term exposure to air pollution has been shown to be associated with a higher risk of hospital admission or emergency department visit for psychiatric disorders, such as mental disorder, depression, schizophrenia, suicide attempt, substance abuse disorder, and panic attack.[34] However, to the best of our knowledge, no direct evidence, except the present study, exists on the association between short-term exposure to air pollution and ASD symptom aggravation.”

34 Bernardini F, Trezzi R, Quartesan R, *et al.* Air Pollutants and Daily Hospital Admissions for Psychiatric Care: A Review. *Psychiatr Serv* 2020;**71**:1270–6. doi:10.1176/appi.ps.201800565

Page 19 (DISCUSSION)

VERSION 2 – REVIEW

REVIEWER	BAGHDADLI, AMARIA CHU of Montpellier, Cente de ressources autisme
REVIEW RETURNED	31-May-2022
GENERAL COMMENTS	The authors did a good job by revising their article. They have clarified the manuscript and answered most of the questions. I thank them for this.
REVIEWER	Bernardini, Francesco Azienda Sanitaria Friuli Occidentale
REVIEW RETURNED	07-Jun-2022
GENERAL COMMENTS	The authors correctly addressed all my comments. I have no further recommendations.