Protein levels, air pollution and vitamin D deficiency: links with allergy

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To the Editor:

The prevalence of allergic diseases has been increasing for several decades. This has been partly attributed to changing environmental factors such as exposure to traffic-related air pollution (TRAP) and nutrient deficiencies, including vitamin D. Furthermore, population-based studies have suggested that air pollution may contribute to vitamin D deficiency [1], while vitamin D levels may modify pollution-driven asthma symptoms in paediatric obesity [2]. Vitamin D supplementation has also been shown to reduce the effects of pollution on asthma and other chronic respiratory diseases [3]. To date, few mechanistic studies have aimed to identify the pathways that may explain these interactions.

Omic approaches are increasingly used to differentiate asthma and allergic disease endotypes, and to identify biomarkers and pathological mediators. To our knowledge, no study has assessed the proteomic response to more than one environmental factor, and their interactions, in the context of asthma and allergy. We therefore assessed the proteome in plasma from a subset of individuals in the Tasmanian Longitudinal Health Study (TAHS) cohort. We aimed to explore the effects of TRAP, using nitrogen dioxide (NO_2) as a proxy, and vitamin D status, on asthma and allergy and investigate the biological mechanisms that underlie these associations.

A subgroup (n=74) of middle-aged adults (mean±SD age 45±1 years) was randomly selected from the 45-year follow-up of the TAHS cohort [4]. As described previously [5], asthma and allergic conditions were defined by questionnaire and skin-prick tests respectively, while exposure to annual outdoor NO_2 concentrations was estimated using a validated satellite-based land-use regression model approach [6]. We measured serum 25-hydroxyvitamin D_3 ($25(OH)D_3$) levels using liquid chromatography–tandem mass spectrometry (LC-MS/MS) and analysed the proteomic profile using Orbitrap LC-MS/MS [7]. We categorised the participants into low or high NO_2 exposure groups using a threshold of 5.1 ppb based on the mean annual NO_2 exposure of the TAHS cohort (5.1±2.6 ppb, n=1367) [5] and we defined vitamin D deficiency as a $25(OH)D_3$ level <50 $nmol \cdot L^{-1}$ [8]. There were no differences in the sex distribution or smoking status between the low and high NO_2 or between the vitamin D replete and deficient groups ($p>0.05$).

Air pollution influences the extent to which solar ultraviolet B radiation reaches the earth's surface and plays an independent role in the development of vitamin D deficiency in populations living in highly polluted areas [9]. However, in our cohort, where the NO_2 levels were relatively low [5], we found no association between air pollution and vitamin D deficiency either using linear regression analysis ($r=-0.091$, $p=0.488$) or by comparing the NO_2 levels between the low and normal vitamin D groups ($p>0.05$) using independent t-tests. However, in line with our previous work [5], we did find an association between high NO_2 levels and an increased risk of mixed grass (OR 6.85, 95% CI 1.22–38.32; $p=0.029$) and rye grass pollen allergy (OR 8.00, 95% CI 1.56–40.99; $p=0.013$) using logistic regression analyses with adjustment for sex and smoking status, but not with asthma or other allergic sensitisation phenotypes such as atopy and house dust mite sensitisation ($p>0.05$). In contrast to NO_2, vitamin D levels were not associated with allergic phenotypes in these middle-aged adults ($p>0.05$), consistent with a previous finding that allergic sensitisation was associated with vitamin D deficiency in children and adolescents, but not adults [10]. Having established the main effect of outdoor air pollution on the onset of allergic diseases, we focussed subsequent analyses on exploring the biological mechanisms of air pollution induced allergy, considering vitamin D as a potential modifying factor.

Shareable abstract (@ERSpublications)
This study provides novel insights into mechanisms of traffic-related air pollution-induced allergy by down-regulation *via* complement regulators (CFI, PROS1 and PLG) and its interaction with vitamin D deficiency *via* the complement inhibitor PLG https://bit.ly/3x0jYOw

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Of the 143 proteins quantified in blood proteomics, 13 were differentially expressed between the low and high exposure NO_2 groups, including two (CD5L, p=0.016; CETP, p=0.042) upregulated and 11 (FCN3, p=0.020; PLG, p=0.022; IGKV1–17, p=0.042; IGKV1–39, p<0.001; IGLV2–11, p=0.023; IGHV3–33, p=0.006; IGKV1–16, p=0.021; CFI, p=0.011; PROS1, p=0.039; CFL1, p=0.037; ACTG1, p=0.004) downregulated in response to high NO_2, with two (PLG and ACTG1) modified by vitamin D levels (interaction p=0.038 and p=0.007 respectively). Stratified analysis of these two proteins by vitamin D status showed that the significant differences were only present in the group with vitamin D deficiency (PLG: mean 0.228, 95% CI −0.287–0.743 for low NO_2 *versus* mean −1.267, 95% CI −2.931–0.397 for high NO_2, p=0.013; ACTG1: mean 0.084, 95% CI −0.429–0.597 for low NO_2 *versus* mean −1.794, 95% CI −3.341–−0.246 for high NO_2, p=0.002). We also identified 23 dysregulated proteins in the subjects with low vitamin D levels (p<0.05), of which five (IGKV1–17, IGLV2–11, IGHV3–33, IGKV1–16 and ACTG1) overlapped with proteins linked to NO_2 exposure. These overlapping proteins were downregulated in the participants exposed to high NO_2 and with low vitamin D (figure 1a).

Of the overlapping proteins, IGKV1–17 expression level was lower in the participants with asthma (mean −0.586, 95% CI −1.175–0.002) than those without asthma (mean 0.082, 95% CI −0.183–0.347) (p=0.042). However, we did not find any link between these proteins and other allergic outcomes. Most of the proteins identified as being differentially expressed are linked to immune regulation, complement activation, phagocytosis or proteolysis (figure 1b). Dysregulation of these proteins may represent common mechanisms through which air pollution and vitamin D deficiency drive adverse health outcomes. Further

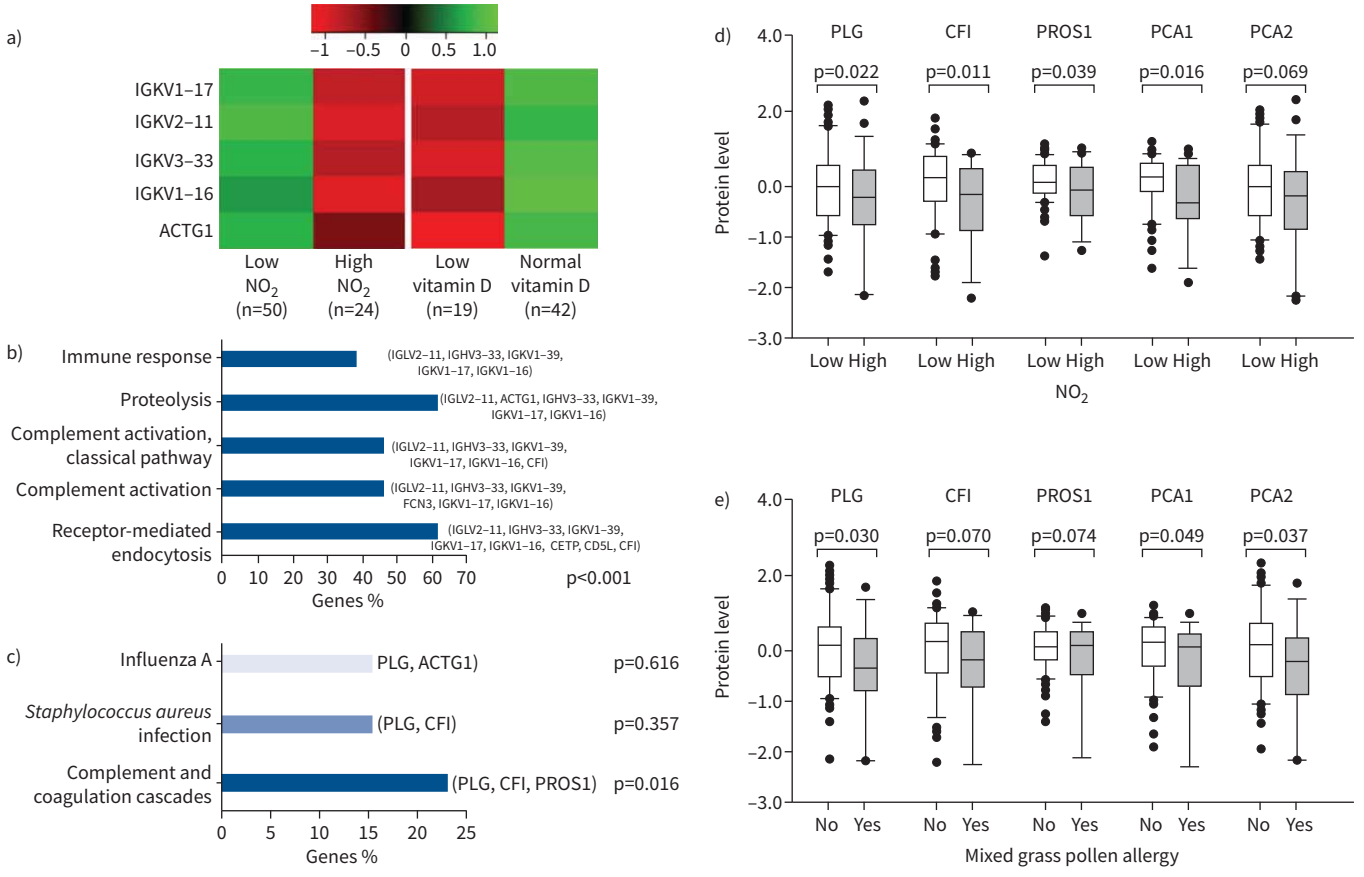

FIGURE 1 a) Heatmap showing expression of the five proteins downregulated in the participants with high NO_2 and low vitamin D levels. b) Gene ontology annotation and c) the Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used to identify significantly enriched biological processes and pathways, with the Benjamini p-value shown. The expression of selected proteins were compared between d) low (n=50) and high NO_2 (n=24) groups, and e) in the individuals with (n=16) and without (n=54) mixed grass pollen (Kentucky bluegrass, orchard, redtop, timothy, sweet vernal grass and meadow fescue) allergy using general linear models, with adjustment for sex and smoking status. Principal component analysis (PCA) was applied to group the correlated proteins in the complement and coagulation cascades, with generation of two scores: PCA1 (56%) and PCA2 (35%). PLG: plasminogen; CFI: complement factor I; PROS1: protein S (alpha).

investigation using a larger sample, including more diverse disease phenotypes, is warranted to link these findings directly to specific phenotypes.

Mapping of the 13 differentially expressed proteins associated with increased NO_2 exposure revealed links to the complement and coagulation cascades (CCC) pathway (figure 1c). This was characterised by downregulation of three proteins, CFI, PLG and PROS1 (figure 1d), with one of these (PLG) modified by vitamin D deficiency. These proteins were also decreased in the participants with atopic responses to mixed grass pollen allergens (figure 1e), indicating an important potential role for these proteins in environmental (TRAP-related) allergy. Uncontrolled activation of CCC is thought to promote the development of Th2-driven allergic inflammation [11], although the exact mechanism is complex and remains elusive.

Given the inhibitory role of these proteins (CFI, PLG and PROS1) in targeting the central components of the C3 and C5 complement cascade, they may act in a coordinated way to activate CCC. For example, CFI is a serine protease which acts as a C3b/C4b inactivator, while C3b is part of the C5 convertases that cleave C5 into anaphylatoxins (C5a) to amplify mast cell degranulation, contributing to increased Type I hypersensitivity [11, 12]. PLG is a complement inhibitor that binds C3, the C3 cleavage products C3b and C3d, and C5, and enhances CFI-mediated C3b degradation [13]. PROS1 is a vitamin K-dependent glycoprotein cofactor to the serine protease that binds to the nascent complement complex C5,6,7 and prevents inappropriate activation of the complement system [14]. From a mechanistic perspective, air pollutants cause oxidative injury to the airways, leading to inflammation, remodelling and increased risk of sensitisation [15], and some studies suggest that oxidative stress mediates complement activation [16]. Therefore, it is of interest to determine whether the identified proteins are regulated by oxidative stress in the context of air pollution and allergy.

Thus, acknowledging the limitation of our sample size, we were able to identify three complement regulators that suggest a novel mechanism *via* activation of the complement system and subsequent development of allergy as a result of exposure to TRAP. Additionally, we were able to identify a potential mechanistic interaction between air pollution and vitamin D *via* the complement inhibitor, PLG. These data highlight the importance of considering interactions between multiple environmental exposures in the context of allergic diseases. Larger-scale molecular studies are warranted to externally validate and further investigate these interactions.

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