



Early View

Research letter

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

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"Take home" message

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.

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.

Oomics-based 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 sub-set 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 age adults (mean 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 D₃ (25(OH)D₃) levels using liquid chromatography-tandem mass spectrometry (LC-MS), and analysed the proteomic profile using Orbitrap LC-MS/MS (7). We categorized the participants into Low or High NO₂ exposure groups using a threshold of 5.1 ppb based on the mean annual NO₂ exposure of the TAHS cohort (5.1 ± 2.6 ppb, n=1367) (5) and we defined vitamin D deficiency as a 25(OH)D₃ level below 50 nmol/L (8). There were no differences in the sex distribution or smoking status between the Low and High NO₂ or between the vitamin D replete and deficient groups ($p>0.05$).

Air pollution influences the extent to which solar ultra-violet 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 NO₂ 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 NO₂ 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 NO₂ 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 gender and smoking status, but not with asthma or other allergic sensitization phenotypes such as atopy and house dust mite sensitization ($p>0.05$). In contrast to NO₂, vitamin D levels were not associated with allergic phenotypes in these middle age adults ($p>0.05$), consistent with a previous finding that allergic sensitization 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, taking into account vitamin D as a potential modifying factor.

Of the 143 proteins quantified in blood proteomics, 13 were differentially expressed between the Low and High exposure NO₂ groups, including 2 (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 NO₂, with 2 (PLG and ACTG1) modified by vitamin D levels (interaction $p=0.038$ and $p=0.007$ respectively). Stratified analysis of these 2 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 NO₂ *versus* mean -1.267, 95% CI -2.931~0.397 for High NO₂, $p=0.013$; ACTG1: mean 0.084, 95% CI -0.429~0.597 for Low NO₂ *versus* mean -1.794, 95% CI -3.341~-0.246 for High NO₂, $p=0.002$). We also identified 23 dysregulated proteins in the subjects with low vitamin D levels ($p<0.05$), of which 5 (IGKV1-17, IGLV2-11, IGHV3-33, IGKV1-16, ACTG1) overlapped with proteins linked to NO₂ exposure. These overlapping proteins were down-regulated in the participants exposed to high NO₂ 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 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 NO₂ exposure revealed links to the complement and coagulation cascades (CCC) pathway (Figure 1c). This was characterised by down-regulation of three proteins including 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 acting 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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Disclosure Statement

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Figure legend

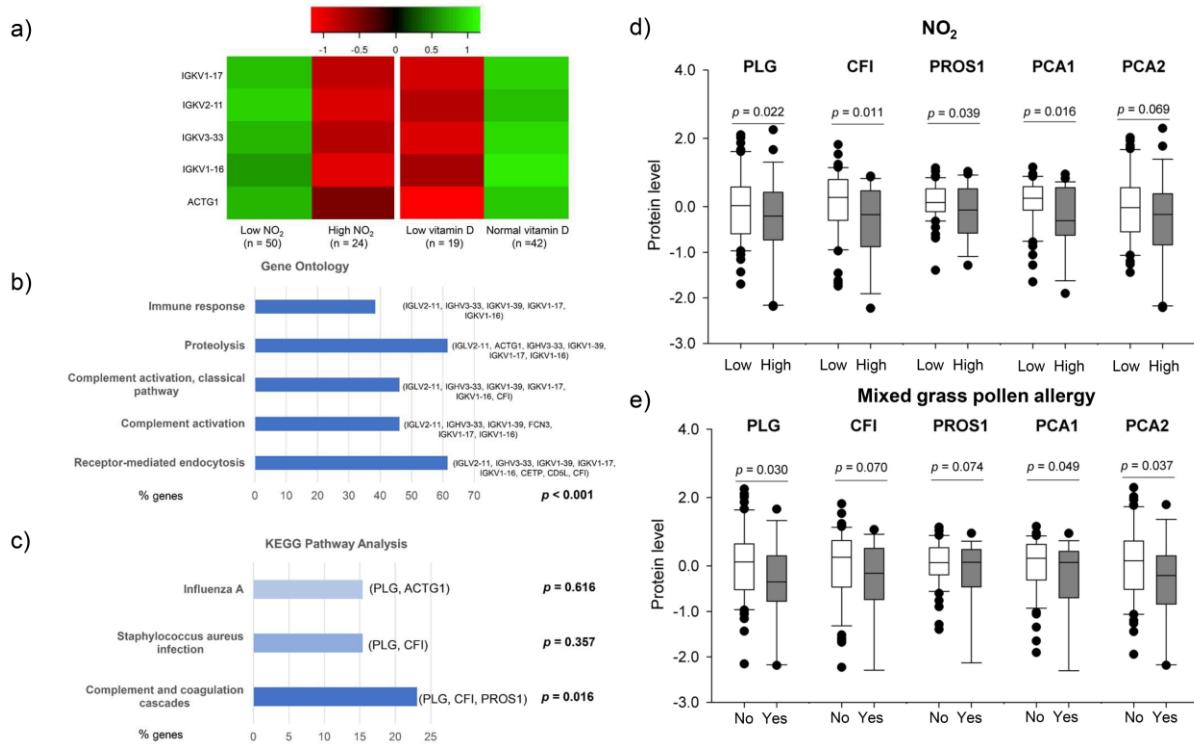


Figure 1: a) Heatmap showing expression of the five proteins down-regulated in the participants with high NO_2 and low vitamin D levels. b-c) Gene Ontology annotation and the KEGG (Kyoto Encyclopedia of Genes and Genomes) database was used to identify significantly biological processes and enriched pathways, with Benjamini p value shown. d- e) The selected protein levels are compared between Low ($n = 50$) and High NO_2 ($n = 24$) groups, and in the individuals with ($n = 16$) and without ($n = 54$) mix allergy using general linear models, with adjustment for gender 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%). Mix: sensitization to mixed grasses (which included Kentucky bluegrass, orchard, redtop, timothy, sweet vernal grass, and meadow fescue). PLG: plasminogen, CFI: complement factor I, PROS1: protein S (alpha).