The role of gene–ambient air pollution interactions in paediatric asthma

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Abstract

Globally, asthma prevention and treatment remain a challenge. Ambient air pollution (AAP) is an environmental risk factor of special interest in asthma research. AAP is poorly defined and has been subdivided either by the origin of the air pollution or by the specific bioactive compounds. The link between AAP exposure and asthma exacerbations is well established and has been extensively reviewed. In this narrative review, we discuss the specific genetic variants that have been associated with increased AAP sensitivity and impact in paediatric asthma. We highlight the relative importance of variants associated with genes with a role in oxidant defences and the nuclear factor-κB pathway supporting a potential central role for these pathways in AAP sensitivity.

Introduction

Asthma remains one of the most significant noncommunicable diseases globally [1, 2]. However, our understanding of asthma pathogenesis and our ability to prevent asthma remain limited. Researchers have identified various lifestyle, infectious, dietary and medical risk factors for asthma [3–7]. Amongst these, ambient air pollution (AAP) was found to be a major risk factor for asthma [8–13]. Decreasing AAP has been shown to be associated with reduced asthma incidence and severity [14, 15]. Despite this, high levels of AAP remain a global problem, with significant associated morbidity and mortality [16]. We currently lack effective treatment strategies to protect paediatric asthma patients from the impact of AAP exposure. Developing these strategies has been complicated by significant heterogeneity in AAP sensitivity. As such, an improved insight into the mechanism at the basis of this heterogeneity is urgently needed.

Variation in AAP sensitivity is likely due in part to genetic variability. We know that, just like environmental factors, asthma risk is modified by genetic variants. In twin studies, the observed heritability for asthma ranges from 35 to 73% [17, 18]. Over the past 25 years, hundreds of genetic variants have been associated with asthma [19–26]. Despite this, the cumulative effect of these variants falls short of the observed heritability [26]. This gap between the large observed heritability and the heritability explained by identified variants has been termed the ‘missing heritability’ [27]. A possible explanation for this missing heritability is that interactions between genetic variants and environmental factors may increase the effect size beyond what can be explained by one factor alone [27].

In this narrative review, we discuss the interactions between genetic variants and AAP in paediatric asthma. In preparing this review, we queried the Medline, Cochrane Library, Scopus and Web of Science databases for publications related to AAP, genetic variants and paediatric asthma. This review is not an attempt to critically appraise the entirety of the published literature. Instead, we aimed to organise the available data and assess the patterns that are starting to emerge, thereby guiding future direction for the field.
First, we will discuss the evidence for a genetic component to AAP sensitivity. Then, we will take a closer look at the specific variants that have been identified to date and the molecular pathways they implicate. Of note, we will not include a discussion regarding the interaction between tobacco smoke, genetic variants and asthma as this has been recently reviewed elsewhere [28]. Similarly, as an excellent recent systematic review examined the interaction between several genetic variants and indoor air pollution, we will not repeat this discussion here [29].

**AAP definition and link to asthma**

While technical limitations and heterogeneous study designs have created different somewhat overlapping categories of AAP, several lines of evidence support its link with asthma. In this section we provide an overview of the different AAP classifications, briefly touch upon the evidence linking AAP to asthma and then discuss the population-level evidence for a genotypical influence on this relationship.

**Types of AAP**

AAP is a broad term that is typically subdivided either by the origin of the air pollution or by the specific bioactive compound that is measured.

In origin-based AAP classifications the effects of a gas mixture emitted by a specific source are evaluated. Examples are ‘traffic-related air pollution’ (TRAP), or ‘secondhand smoke’ also known as ‘environmental tobacco smoke’. The difficulty with this approach is that these categories encompass several bioactive components. The specific components of these mixtures are not necessarily consistent or reproducible. Conversely, an inherent advantage is that it captures possible interaction effects between different components. Studies assessing TRAP typically use some surrogate marker such as reported truck traffic on the street of residence or measured nitrogen dioxide (NO2) [30, 31]. Unfortunately, these surrogate markers come with their own limitations and confounders [32].

Studies looking directly at specific bioactive compounds have generally measured benzo[a]pyrene (B[a]P), carbon monoxide (CO), lead, NO2, ozone (O3), particulate matter (PM) and/or sulphur dioxide (SO2) [33–35]. While this approach allows for more exact and reproducible measurements, distinguishing association from causation remains complex. For example, when SLAUGHTER et al. [36] found a significant association between CO and asthma symptom severity, this was hypothesized to be due to the effect of nonmeasured combustion byproducts.

Compounds of particular interest in paediatric asthma research have been NO2, O3 and PM.

NO2 is a combustion byproduct and was initially viewed as mostly an indoor air pollutant [34]. However, since up to 80% of ambient NO2 is caused by traffic, it has been used as a proxy for TRAP [9, 30]. A limitation of this approach is that while ambient NO2 is mostly caused traffic, its specificity will be decreased in areas with other sources of exhaust fumes as it is a byproduct of all types of exhaust [32]. Increased NO2 levels as measured outside children’s homes have been associated with increased new-onset asthma [30].

O3 is formed by an interaction between ultraviolet light and precursor compounds. It is most prevalent in areas with stagnant weather patterns and prolonged sunlight [37]. Several O3 precursors are found in industrial and vehicle exhaust [9, 37]. Increased O3 has been associated with increased asthma medication usage and morbidity [38–41]. Beyond this, higher mean 4-year O3 concentrations have also been associated with increased asthma diagnosis in children engaged in sports [42].

PM is a heterogeneous group that is typically subdivided based on the size of particulates. PM10 and PM2.5 refer to particulates with an aerodynamic diameter below 10 and 2.5 μm, respectively [8, 34]. This group includes fine solids, dust, aerosols, soot, ash and pollen [37]. There is significant temporal and spatial variation in the content of PM [34]. PM can encompass compounds of specific interest such as polyaromatic hydrocarbons including B[a]P and diesel soot or diesel exhaust particulate (DEP) [37, 43]. PM is sometimes subdivided by pH and acidic PM was specifically associated with decreased lung function in children [44]. Both PM10 and PM2.5 exposure have been associated with increased asthma symptom frequency and decreased asthma control [8].

**Link between AAP and asthma**

The link between AAP exposure and asthma exacerbations is well established and has been extensively reviewed [8, 9]. This association remained significant in a meta-analysis [10].
Whether AAP causes asthma, however, has been a topic of discussion [45]. While an epidemiological link has been evident for years, it is only recently that evidence has supported a causal link. This link was reviewed by both Burbank et al. [11] and Hehua et al. [12] and was further supported in a recent systemic review by Khreis et al. [13].

As data highlighting the role of AAP in both asthma severity and incidence became available, differences in AAP sensitivity between ethnic groups became apparent. In 2014, Wendt et al. [46] noted a significantly higher effect of increased O₃ levels on black patients as opposed to white patients with asthma. Recently, Native American ancestry was also shown to interact with AAP and influence response to bronchodilator therapy [47]. This increased sensitivity likely contributes to the high asthma burden of disease in these populations. While differences in environmental exposures likely play a role, a genetic modification of the effect of air pollution was suggested as far back as 2004 [37]. Of note, environmental and genetic factors may also work synergistically. For example, as we will describe further in the article, many genetic variants associated with oxidative stress are related to AAP sensitivity. Socio–economic factors leading to a diet poor in antioxidants could logically further predispose at-risk populations. Beyond cohorts based on ethnicity, differences in AAP sensitivity in cohorts based on ancestral atopic disease status have been described in France [48]. This observation seems to support the notion that the observed differences are in part due to genetic differences and not solely due to differences in environmental factors secondary to socio–economic status.

Specific pathways linked to air pollution sensitivity
Here, we discuss the specific genetic variants that have been linked to AAP sensitivity. An overview of the implicated genes and associated pathways is presented in figure 1 while table 1 summarises the genetic variants and their effect. In our discussion, we start with the genes that control antioxidant defences, then move to those that control the immune response, genes involved in airway development and repair, and conclude with genes controlling bronchial responsiveness.

**FIGURE 1** Overview of genes and pathways related to pulmonary oxidative stress implicated in ambient air pollution sensitivity. Genes associated with variants that impact ambient air pollution sensitivity are in green ovals and biological processes marked in red rectangles. ADCY2: adenylate cyclase 2; ARG1: arginase 1; B4GALT4: beta-1,4-galactosyltransferase 1; CAT: catalase; CTLA4: cytotoxic T-lymphocyte associated protein 4; CYP2E1: cytochrome P450 2E1; GSTM1: glutathione S-transferase Mu 1; GSTP1: glutathione-S-transferase Pi 1; HMOX-1: heme oxygenase 1; MPO: myeloperoxidase; NLRP3: NOD-, LRR- and pyrin domain-containing protein 3; NF-κB: nuclear factor κB; STAT4: signal transducer and activator of transcription 4; TGF-B1: transforming growth factor B1; TLR: Toll-like receptor; TNF-α: tumour necrosis factor α; TXNDC8: thioredoxin domain containing 8.
### TABLE 1 Overview of the published variants, the implicated pathways and the described effect

| Pathway                      | Protein family     | Associated gene | Variant (associated SNP if reported) | Functional impact                          | Effect in interaction with AAP                                      | Reference                          |
|------------------------------|--------------------|-----------------|--------------------------------------|------------------------------------------|---------------------------------------------------------------------|-----------------------------------|
| **Antioxidant defences**     | Glutathione-S-transferase | GSTM1           | Null genotype                        | No functional protein                    | Significant reductions in FEF25–75% after ozone exposure             | ROMIEU et al. [58]                |
|                              |                    | GSTP1           | I/I at codon 105 (rs947894)          | Change in catalytic properties           | Higher childhood asthma incidence for those living in a high air pollution district (adjusted OR 5.52, CI 1.64–21.25). Higher risk for childhood asthma for those living in a community with low PM2.5 concentrations (no effect size reported). | Lee et al. [59]                   |
|                              |                    |                 | I/V or V/V at codon 105 (rs947894)   | Change in catalytic properties           | Negative association between PM2.5 and ozone and childhood asthma (adjusted OR 0.6, CI 0.45–0.82 and 0.74, CI 0.6–0.9, respectively). | Su et al. [60]                    |
| **NAD(P)H dehydrogenase 1** | NQO1               | At least one S at codon 187† | Lower enzymatic activity             | Significantly reduced asthma risk as opposed to P/P homozygotic subjects (risk ratio 0.4, CI 0.2–0.8). | Salam et al. [65]                                                   |
| **Microsomal EPH**           | EPHX1              | Y at codon 113 and R at codon 139¶ | Higher enzymatic activity            | Increased risk for asthma in children with high enzymatic activity living near a major road (risk ratio 3.2, CI 1.75–6.00). | Salam et al. [65]                                                   |
| **Catalase**                 | CAT                | G/G at codon 330 (rs1001179)† | Decreased enzymatic activity         | Increased respiratory-related school absences in communities with high NO2 (risk ratio 1.53 with CI 1.09–2.14). | Wenten et al. [66]                                                  |
| **Myeloperoxidase**          | MPO                | G/A or A/A at codon 463 (rs2333227)† | Decreased enzymatic activity         | Increased respiratory related school absences in communities with high NO2 (risk ratio 1.53 with CI 1.09–2.14). | Wenten et al. [66]                                                  |
| **Heme oxygenase-1**         | HMOX-1             | Fewer than 23 (GT)n repeats | Decreased inducibility                | Reduced risk for now-onset asthma in non-Hispanic white children (HR 0.64, CI 0.41–0.99). | Islam et al. [68]                                                   |
| **Thioredoxin reductase**    | TXNDC8             | rs7041938 T>G   | Not described                        | Significant interaction with NO2 exposure in Caucasian children with asthma (no effect size reported). | Ierodiakonou et al. [70]                                            |

Continued
| Pathway                        | Protein family | Associated gene | Variant (associated SNP if reported) | Functional impact | Effect in interaction with AAP                                                                 | Reference          |
|-------------------------------|----------------|-----------------|--------------------------------------|-------------------|------------------------------------------------------------------------------------------------|--------------------|
| Intrinsic oxidative load      | Cytochrome P450 2E1 protein | CYP2E1         | rs2070673 AT or TT genotype          | Not described     | Increased odds for asthma in children living in an area with high B[a]P exposure (OR 5, CI 3.1–8.8). | Choi et al. [35]   |
|                               | B4GALT5        | B4GALT5        | rs686237 A>C                         | Increased expression | Significant interaction with NO₃ at birth address and childhood asthma (no effect size reported). | Gref et al. [72]   |
| Nitrosative stress            | Arginase       | ARG1            | rs2749935 A>T                         | Not described     | Reduced asthma risk amongst atopic children in high ozone communities (OR per haplotype copy 0.12, CI 0.04–0.43). | Salam et al. [76]  |
| Immune response               | Tumour necrosis factor | TNF-α         | G/G at position 308 of the TNF-α gene promoter¶ | Decreased expression | Decreased wheezing incidence in children living in low ozone communities (OR 0.5, CI 0.4–0.7). | Li et al. [82]     |
|                               | Toll-like receptor | TLR-2           | rs4696480 T>A                        | Not described     | Increased risk for asthma with increasing PM₂.₅ exposure (OR 2.0, CI 1.2–3.1 for every interquartile range increase). | Kerkhof et al. [83] |
|                               |                 | TLR-4           | rs2770150 TC; rs10759931 GG; rs6476317 GG; rs10759932 CT or CC; rs1927911 TT | Not described     | Increased risk for asthma with increasing PM₂.₅ exposure (OR 2.0, CI 1.1–3.6; OR 2.6, CI 1.4–4.9; OR 2.2, CI 1.2–4.3; OR 2.9, CI 1.2–6.9; OR 4.4, CI 1.7–11.7). | Kerkhof et al. [83] |
|                               | Cytotoxic       | CTLA4           | rs11571316 G>A; rs11571319 G>A       | Suspected decreased enzymatic activity | Increased odds for asthma in children living in an area with high B[a]P exposure (OR 9, CI 4.56–18.36 and OR 8, CI 3.95–14.2, respectively). | Choi et al. [35]   |
|                               | T-lymphocyte-associated protein 4 |       |                                       |                    |                                                                                                  |                    |
|                               | Signal transducer and activator of transcription 4 | STAT4     | rs1031509 TG or GG                   | Not described     | Increased odds for asthma in children living in an area with high B[a]P exposure (OR 5, CI 3.03–8.55). | Choi et al. [35]   |
|                               | Eph receptor    | EPHA3           | rs13090972 T>G; rs958144 C>T         | Not described     | Significant interaction with NO₂ exposure in Caucasian children with asthma.                      | Ieroudimou et al. [70] |
| Repair and remodelling        | Transforming growth factor B1 | TGF-B1         | T/T at codon 509 (rs4803457)         | Not described     | Higher risk for early persistent asthma in children with this genotype living near a highway (risk ratio 3, CI 1.29–7.44). | Salam et al. [88]  |
| Adenylyl cyclase type 2       | TGF-B1         | ADcy2           | rs6886921 C>T                        | Not described     | Significant interaction with NO₂ at birth address and childhood asthma.                          | Gref et al. [72]   |

AAP: ambient air pollution; B4GALT4: beta-1,4-galactosyltransferase 1; CI: confidence interval; EPHX1: epoxide hydrolase 1; FEF₂₅–₇₅%: forced mid-expiratory flow at 25–75% of forced vital capacity; GSTM1: glutathione S-transferase Mu 1; GSTP1: glutathione-S-transferase Pi 1; HR: hazard ratio; NAD(P)H: nicotinamide adenine dinucleotide phosphate; OR: odds ratio; PM: particulate matter; SNP: single nucleotide polymorphism. §: reported effect in patients with GSTM1 null genotypes; ¶: reported effect in patients with GSTP1 V/V genotypes; #: reported effect in patients with variants in both genes; †: effect decreased in patients with GSTM1 null or GSTP V at codon 105 genotype.
Genes controlling antioxidant defences

Oxidative stress is defined as an imbalance between oxidants and antioxidant systems with excessive oxidant availability [49]. AAP is known to induce oxidants and increase oxidative stress. This is thought to be one of the major pathways by which AAP influences asthma [50, 51].

The specific oxidising systems in the lung and the potential role of air pollution in shifting the oxidative balance has been extensively reviewed elsewhere [52]. Here, we will focus on how genetic variants that weaken the response to oxidative stress seem to increase the sensitivity to AAP in asthma. These appear to be localised along one of five antioxidant pathways.

First, the glutathione pathway is likely the best studied in the context of AAP sensitivity. Glutathione-S-transferase (GST) enzymes conjugate reactive oxygen species with reduced glutathione to facilitate their elimination [53, 54]. The cytosolic GST enzymes are subdivided into α, μ, π, σ and θ classes based on their isoform [54]. Regarding the effect of AAP, the θ and μ members of the GST family, encoded by GST Pi 1 (GSTP1) and GST Mu 1 (GSTM1), respectively, have been of particular interest [54]. This is both due to their relative importance in the lung, with GSTP1 estimated to provide up to 90% of pulmonary GST activity, and the relatively high frequency of GSTP1 or GSTM1 null genotypes [55].

The GSTM1 null genotype was shown to be associated with increased markers of oxidative stress after ozone exposure and facilitates DEP-induced allergic inflammation in adults [56, 57]. Specifically in paediatric asthma, ROMIEU et al. [58] showed patients with this genotype experienced significant decreases in forced mid-expiratory flow at 25–75% of forced vital capacity when exposed to high O₃ concentrations whereas others did not.

The results of specific variants within these genes have been more heterogeneous. Up to 40% of the population has two isoleucines as opposed to valine in GSTP1 codon 105 (genotype GSTP1 I/I) [57]. The GSTP1 I/I genotype is associated with DEP-induced allergic inflammation in adults and increased paediatric asthma [57, 59, 60]. Conversely, NOₓ exposure led to allergic sensitisation only in patients with genotype GSTP1 I/V or V/V but not in those with GSTP1 I/I [55]. Furthermore, there was a negative association between asthma, O₃ and PM₂.₅ exposure for GSTP1 I/I homozygotes as opposed to a positive association for those with a GSTP1 I/V or V/V genotype [61]. Finally, in high PM₁₀ exposure communities, GSTP1 I/V or V/V was a risk factor for asthma [60]. We currently lack a clear explanation for these seemingly contradictory findings related to these specific GSTP1 variants.

Two possible explanations for this observed heterogeneity have been described. On one side, we know that the components of PM have a wide spatio–temporal variation. Since these variants change the catalytic properties of the GST enzymes, it is possible that the effect of the variant is dependent on the specific PM components to which a patient is exposed [62]. On the other, gene–gene interactions may complicate the observed gene–environment relationship. For example, variants within the nicotinamide adenine dinucleotide plus hydrogen (NAD(P)H) quinone oxidoreductase 1 (NQO1) gene modulate the asthma risk associated with a GSTM1 null genotype [63, 64]. This gene encodes NAD(P)H dehydrogenase 1, a phase 2 detoxification reaction protein that helps protect against damage from oxidative stress.

A further example of a gene–environment interaction can be found when looking at variants within epoxide hydrolase 1 (EPHX1). This gene encodes the microsomal EPH, an important class of enzymes involved in detoxification. These variants impacted the risk of early persistent asthma only in children with a GSTM1 null genotype that lived within 75 m of a major road [65].

The second and third antioxidant pathways involved in AAP are the catalase (encoded by CAT) and myeloperoxidase (encoded by MPO) pathways. These enzymes catalyse the reduction of hydrogen peroxide (H₂O₂). Children with variants in both the promoter region of the CAT and MPO genes were found to be more likely to be absent from school due to respiratory illness when exposed to AAP [66].

The fourth pathway is the heme oxygenase-1 (HO-1 encoded by HMOX-1) pathway [67]. By catalysing heme, HO-1 creates both biliverdin and bilirubin which in turn act as scavengers of reactive oxygen species [67]. HMOX-1 is induced by oxidative stress and this inducibility is inversely correlated to the number of (GT)n repeats. Non-Hispanic white children with fewer than 23 (GT)n repeats were found to be at a reduced risk for asthma. This effect was more pronounced for children living in a low-O₃ community. It was hypothesised that the difference in promoter activity may allow children to respond more optimally to temporary increases in oxidative stress but that this effect is less significant if the antioxidant defences are chronically stimulated by high background O₃ concentrations [68].
The fifth pathway is centred around thioredoxin. Reduced thioredoxin can catalyse the reduction of oxidised cellular proteins. It in turn is then reduced again by thioredoxin reductase. Deficiencies within this system have been associated with COPD, asthma and lung injury [69]. A recent genome wide interaction study linked variants within thioredoxin domain containing 8 (TXNDC8), a gene encoding one of the thioredoxin reductase enzymes, to sensitivity to NO₂ in Caucasians [70].

Aside from variants within the oxidative stress defences, two variants that expose the host to increased intrinsic oxidative stress also increase the sensitivity to AAP. First, a variant within cytochrome P450 2E1 (encoded by CYP2E1) increased air pollution sensitivity [35]. This enzyme plays a central role in detoxification reactions and is known to increase oxidative stress [71]. Second, a variant leading to increased expression of B4GALT5 significantly modified the effect of traffic related AAP on asthma in children [72]. This gene encodes the enzyme B4GALT5 involved in the synthesis of lactosylceramide. The lactosylceramide-centric signalling pathways increase oxidative stress when activated [73].

Closely related to oxidative stress is nitrosative stress. Nitrosative stress is defined as an overproduction of NO free radicals [74]. These can react with reactive oxygen species to form reactive nitrogen species (RNS). Both NO and RNS have been shown to play a significant role in allergic asthma [75]. Arginase, encoded by ARG1 and ARG2, competes with NO synthase and decreases the nitrosative stress. Variants within both ARG1 and ARG2 are associated with asthma but the relationship between variants within ARG1 and asthma was found to be modulated by ozone exposure [76].

There has been significant research interest in modulating oxidative stress in asthma. The results of both paediatric and adult studies were recently expertly reviewed by Sahiner et al. [77]. In brief, the current literature is heterogeneous with inconsistent results of anti-oxidant supplementation. While a study by Romieu et al. [78] analysed subjects by ozone exposure, to our knowledge, no studies have stratified patients by AAP exposure and genetic profile.

**Genes controlling the immune response**

Beyond variants in genes directly related to antioxidant defences, variants in genes controlling the response of the immune system have also been found to interact with AAP. Most of the variants described to date are closely associated with the nuclear factor κB (NF-κB) pathway. NF-κB is a family of nuclear transcription factors that functions as a central pro-inflammatory regulator in both the innate and adaptive immune system [79]. When activated, NF-κB upregulates the transcription of inflammatory proteins and can regulate cell differentiation and proliferation [79]. One of NF-κB’s downstream effectors, the NOD-, LRR- and pyrin domain-containing protein 3 inflammasome, has been suggested as a possible target in severe asthma [80]. Mitochondrial damage, secretion of tumour necrosis factor α (TNF-α) and activation of Toll-like receptors (TLRs) can lead to activation of the NF-κB pathway.

TNF-α is a pro-inflammatory cytokine that plays a role in the innate immune response [81]. It is primarily released by macrophages and leads to phosphorylation of NF-κB. Dysregulation of this pathway has been implicated in asthma and is of particular interest in refractory asthma [81]. With regards to the interaction between AAP and asthma, patients with variants associated with the TNF-α gene were found to be protected from asthma if they grew up in a low O₃ exposure community [82]. The authors speculated that this variant may dampen the inflammatory response to lower doses of oxidative stress [82]. The TLR-2 and TLR-4 receptors bind to either Gram-positive bacteria, mycoplasma, yeast and spirochetes or Gram-negative bacteria, respectively. Variants within these genes were shown to significantly modulate the risk for doctor-diagnosed asthma in children exposed to PM₂·₅ [83].

As noted above, NF-κB also plays a central role in T-cell activation and differentiation [79]. Paradoxically, besides leading to T-cell activation, NF-κB activation also stimulates differentiation of regulatory T-cells (Treg). These Tregs play a central role in controlling the immune response and preventing chronic inflammation [79]. This dual action is of specific interest within asthma as excessive activation of type 2 helper T-cells (Th2 cells) and insufficient downregulation by Treg cells is thought to play a central role in allergic asthma [84]. Unsurprisingly, variants within genes that affect the balance between Treg and Th2 cells have been associated with increased AAP sensitivity. Cytotoxic T-lymphocyte-associated protein 4 (CTLA4, encoded by CTLA4) has a Treg-cell mediated inhibitory effect on T-cells [85]. Variants within CTLA4 leading to decreased CTLA4 activity seemed associated with increased ambient B[a]P asthma risk [35]. Signal transducer and activator of transcription 4 (encoded by STAT4) is another protein with a central role in T-cell differentiation [35]. Variants within STAT4 have been shown to increase the odds for asthma in patients exposed to high levels of AAP [35].
Finally, IERODIAKONOU et al. [70] identified variants within EPHA3 in their NO2 genome-wide interaction study. This gene encodes an Eph receptor and member of the family of tyrosine kinases. This receptor is known to play a role in cell–cell interactions and has been studied mostly in an oncogenic context [86]. To date, its potential role in asthma remains unclear.

It is worth noting that many of the proteins associated with these variants have been targeted either in asthma or other inflammatory processes. For example, due to interesting experimental and animal model results, TNF-α blockade has been an area of extensive research in paediatric asthma [87]. Unfortunately, the results of clinical studies have been mixed. Given the data available from genetic studies, one wonders if these mixed results are partially due to different exposures and genetic susceptibilities in the populations studied.

**Other implicated variants**

Variants in genes with a role in airway development and repair and bronchial responsiveness have also been associated with AAP sensitivity. Children homozygous for threonine as opposed to cysteine in the 508 position of TGF-B1 were found to be at increased risk for asthma if they resided close to a major roadway. This variant is known to increase transforming growth factor B1 (TGF-B1) expression [88]. The transforming growth factor superfamily proteins serve a wide array of functions including regulating cell growth, proliferation and apoptosis [89]. These proteins are secreted in an inactive form and rely on external stimuli, such as oxidative stress, for their activation [89]. Specifically in asthma, TGF-B1 increases smooth muscle proliferation and airway remodelling. Furthermore, TGF-B1 helps to regulate the balance between T-cell populations and is required to maintain peripheral Treg cells [89].

Finally, variants associated with ADCY2 were shown to significantly interact with NO2 on asthma risk [72]. ADCY2 encodes adenylyl cyclase type 2, a receptor expressed on airway smooth muscle cells [90]. Activity of the adenylyl cyclases is upregulated by stimulation of the B-adrenergic receptor and causes smooth muscle relaxation [90].

**Conclusion**

Despite the high global burden of AAP exposure in paediatric asthma, we currently have no effective therapies to protect our patients. Understanding and predicting individual AAP sensitivity is a prerequisite for developing these therapies. To this end, we have tried to organise the current genetic evidence linking specific genetic variants to AAP sensitivity. The bulk of the variants identified to date appear to follow the model put forth by Li et al. [64]. In this model, cells initially respond to oxidative stress by upregulating the antioxidant defences. When these fail and tissue damage occurs, inflammation mediated by the NF-κB pathway follows. However, as highlighted in figure 1, even in the absence of oxidative stress, AAP can worsen asthma via direct activation of the immune system or irritation of the airways. A key next challenge will be the development of sensitive and specific biomarkers for both AAP exposure and the consequences of this exposure. First, such biomarkers could support the biological plausibility of causation as opposed to association. Second, if causation is supported, they could allow us to rapidly identify and target those patients most at risk for developing AAP related morbidity. Finally, they could be incorporated in future clinical trials to guard against inadvertently biasing results. Given the genetic evidence laid out in this review, markers of oxidative stress, airway remodelling and NF-κB mediated inflammation with associated abnormal T-cell differentiation may be especially valuable. Lastly, medications targeting proteins implicated by genetic evidence have been estimated to be roughly twice as likely to make it to the market [91]. As such, proteins discussed in this review may be especially promising therapeutic targets.

**Points for clinical practice and directions for future research**

- While the role of AAP in paediatric asthma severity and incidence has been established, we are only beginning to understand the complexities of individual sensitivity.
- Genetic evidence implicates pulmonary oxidative stress defences and the NF-κB pathway as playing roles in the sensitivity to AAP in paediatric asthma.
- Identifying reliable and reproducible biomarkers of AAP exposure and sensitivity would allow us to prioritise therapeutic and policy interventions.

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References
1. Global Asthma Network. The Global Asthma Report 2018. http://globalasthmareport.org/resources/Global_Asthma_Report_2018.pdf Date last updated: 2018.
2. Yaghoubi M, Adibi A, Safari A, et al. The projected economic and health burden of uncontrolled asthma in the United States. Am J Respir Crit Care Med 2019; 200: 1102–1112.
3. Dharmage SC, Perret JL, Custovic A. Epidemiology of asthma in children and adults. Front Pediatr 2019; 7: 246.
4. Beasley R, Semprini A, Mitchell EA. Risk factors for asthma: is prevention possible? Lancet 2015; 386: 1075–1085.
5. Strachan DP, Cook DG. Health effects of passive smoking. 6. Parental smoking and childhood asthma: longitudinal and case-control studies. Thorax 1998; 53: 204–212.
6. Tatum AJ, Shapiro GG. The effects of outdoor air pollution and tobacco smoke on asthma. Immunol Allergy Clin North Am 2005; 25: 15–30.
7. Huang YJ, Boushey HA. The microbiome in asthma. J Allergy Clin Immunol 2015; 135: 25–30.
8. Guarnieri M, Balmes JR. Outdoor air pollution and asthma. Lancet 2014; 383: 1581–1592.
9. Tirotu AI, Novakova P, Nedeva D, et al. Impact of air pollution on asthma outcomes. Int J Environ Res Public Health 2020; 17: 6212.
10. Orellano P, Guaranta N, Reynoso J, et al. Effect of outdoor air pollution on asthma exacerbations in children and adults: systematic review and multilevel meta-analysis. PLoS One 2017; 12: e0174050.
11. Burbank AJ, Sood AK, Kecic MJ, et al. Environmental determinants of allergy and asthma in early life. J Allergy Clin Immunol 2017; 140: 1–12.
12. Hehua Z, Qing C, Shanyan G, et al. The impact of prenatal exposure to air pollution on childhood wheezing and asthma: a systematic review. Environ Res 2017; 159: 519–530.
13. Khreis H, Kelly C, Tate J, et al. Exposure to traffic-related air pollution and risk of development of childhood asthma: a systematic review and meta-analysis. Environ Int 2017; 100: 1–31.
14. Garcia E, Berhane KT, Islam T, et al. Association of changes in air quality with incident asthma in children in California, 1993–2014. JAMA 2019; 321: 1906–1915.
15. Guerriero C, Chatzidiakou L, Cairns J, et al. The economic benefits of reducing the levels of nitrogen dioxide (NO2) near primary schools: the case of London. J Environ Manage 2016; 181: 615–622.
16. World Health Organization. Ambient air pollution: A global assessment of exposure and burden of disease. https://apps.who.int/iris/handle/10665/250141 Date last updated: 2016.
17. Polderman TJ, Benyamin B, de Leeuw CA, et al. Meta-analysis of the heritability of human traits based on fifty years of twin studies. Nat Genet 2015; 47: 702–709.
18. Thomsen SF, Duffy DL, Kyvick KD, et al. Genetic influence on the age at onset of asthma: a twin study. J Allergy Clin Immunol 2010; 126: 626–630.
19. Daniels SE, Bhattacharrya S, James A, et al. A genome-wide search for quantitative trait loci underlying asthma. Nature 1996; 383: 247–250.
20. Hakonarson H, Halapi E. Genetic analyses in asthma: current concepts and future directions. Am J Pharmacogenomics 2002; 2: 155–166.
21. Moffatt MF, Kabesch M, Liang L, et al. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. Nature 2007; 448: 470–473.
22. Moffatt MF, Gut IG, Demenais F, et al. A large-scale, consortium-based genomewide association study of asthma. N Engl J Med 2010; 363: 1211–1221.
23. Sleiman PM, Hakonarson H. Recent advances in the genetics and genomics of asthma and related traits. Curr Opin Pediatr 2010; 22: 307–312.
24. March ME, Sleiman PM, Hakonarson H. The genetics of asthma and allergic disorders. Discov Med 2011; 11: 35–45.
53 Piacentini S, Polimanti R, Simonelli I, et al. Glutathione S-transferase polymorphisms, asthma susceptibility and confounding variables: a meta-analysis. Mol Biol Rep 2013; 40: 3299–3313.
54 To-Figueras J. Glutathione S-transferase M1 (GSTM1) and T1 (GSTT1) polymorphisms and lung cancer risk among Northwestern Mediterraneans. Carcinogenesis 1997; 18: 1529–1533.
55 Melén E, Nyberg F, Lindgren CM, et al. Interactions between glutathione S-transferase P1, tumor necrosis factor, and traffic-related air pollution for development of childhood allergic disease. Environ Health Perspect 2008; 116: 1077–1084.
56 Corradi M, Alinovi R, Goldoni M, et al. Biomarkers of oxidative stress after controlled human exposure to ozone. Toxicol Lett 2002; 134: 219–225.
57 Gilliland FD, Li Y-F, Saxon A, et al. Effect of glutathione-S-transferase M1 and P1 genotypes on xenobiotic enhancement of allergic responses: randomised, placebo-controlled clinical study. Lancet 2004; 363: 119–125.
58 Romieu I, Siena-Monge JJ, Ramírez-Aguilar M, et al. Genetic polymorphism of GSTM1 and antioxidant supplementation influence lung function in relation to ozone exposure in asthmatic children in Mexico City. Thorax 2004; 59: 8–10.
59 Lee YL, Lin YC, Lee YC, et al. Glutathione S-transferase P1 gene polymorphism and air pollution as interactive risk factors for childhood asthma. Clin Exp Allergy 2004; 34: 1707–1713.
60 Su MW, Tsai CH, Tung KY, et al. GSTP1 is a hub gene for gene–air pollution interactions on childhood asthma. Allergy 2013; 68: 1614–1617.
61 Hwang B-F, Young L-H, Tsai C-H, et al. Fine particle, ozone exposure, and asthma/wheezeing: effect modification by glutathione S-transferase P1 polymorphisms. PLoS One 2013; 8: e52715.
62 Sundberg K. Differences in the catalytic efficiencies of allelic variants of glutathione transferase P1-1 towards carcinogenic diol epoxides of polycyclic aromatic hydrocarbons. Carcinogenesis 1998; 19: 433–436.
63 David GL, Romieu I, Siena-Monge JJ, et al. Nicotinamide adenine dinucleotide (phosphate) reduced: quinone oxidoreductase and glutathione S-transferase M1 polymorphisms and childhood asthma. Am J Respir Crit Care Med 2003; 168: 1199–1204.
64 Li N, Hao M, Phalen RF, et al. Particulate air pollutants and asthma. Clin Immunol 2003; 109: 250–265.
65 Salam MT, Lin PC, Avol EL, et al. Microsomal epoxide hydrolase, glutathione S-transferase P1, traffic and childhood asthma. Thorax 2007; 62: 1050–1057.
66 Wenten M, Gauderman WJ, Berhane K, et al. Functional variants in the catalase and myeloperoxidase genes, ambient air pollution, and respiratory-related school absences: an example of epistasis in gene-environment interactions. Am J Epidemiol 2008; 170: 1494–1501.
67 Tanaka G, Aminuddin F, Akhabir L, et al. Effect of heme oxygenase-1 polymorphisms on lung function and gene expression. BMC Med Genet 2011; 12: 117.
68 Islam T, McConnell R, Gauderman WJ, et al. Ozone, oxidant defense genes, and risk of asthma during adolescence. Am J Respir Crit Care Med 2008; 177: 386–395.
69 Xu J, Li T, Wu H, et al. Role of thioredoxin in lung disease. Pulm Pharmacol Ther 2012; 25: 154–162.
70 Ierodiakonou D, Coull BA, Zanobetti A, et al. Pathway analysis of a genome-wide gene by air pollution interaction study in asthmatic children. J Exp Sci Environ Epidemiol 2019; 29: 539–547.
71 Gonzalez FJ. Role of cytochromes P450 in chemical toxicity and oxidative stress: studies with CYP2E1. Mutation Res 2005; 569: 101–110.
72 Gref A, Merid SK, Gruzieva O, et al. Genome-wide interaction analysis of air pollution exposure and childhood asthma with functional follow-up. Am J Respir Crit Care Med 2017; 195: 1373–1383.
73 Chatterjee S, Balram A, Li W. Convergence: lactosylceramide-centric signaling pathways induce inflammation, oxidative stress, and other phenotypic outcomes. Int J Mol Sci 2021; 22: 1816.
74 Ganten D, Ruckpaul K, Birchmeier W, et al. eds. Nitrosative stress. In: Encyclopedic Reference of Genomics and Proteomics in Molecular Medicine. Berlin, Heidelberg, Springer Berlin Heidelberg, 2006; p. 1293.
75 Zuo L, Koozechian MS, Chen LL. Characterization of reactive nitrogen species in allergic asthma. Ann Allergy Asthma Immunol 2014; 112: 18–22.
76 Salam MT, Islam T, Gauderman WJ, et al. Roles of arginase variants, atopy, and ozone in childhood asthma. J Allergy Clin Immunol 2009; 123: 596–602.e6.
77 Sahiner UM, Birben E, Erzurum S, et al. Oxidative stress in asthma: part of the puzzle. Pediatr Allergy Immunol 2018; 29: 789–800.
78 Romieu I, Siena-Monge JJ, Ramírez-Aguilar M, et al. Antioxidant supplementation and lung functions among children with asthma exposed to high levels of air pollutants. Am J Respir Crit Care Med 2002; 166: 703–709.
79 Liu T, Zhang L, Joo D, et al. NF-κB signaling in inflammation. Signal Transduct Target Ther 2017; 2: 17023.
80 Theofani E, Semitekolou M, Morianos I, et al. Targeting NLRP3 inflammasome activation in severe asthma. J Clin Med 2019; 8: 1615.
81 Berry M, Brightling C, Pavord I, et al. TNF-α in asthma. Curr Opin Pharmacol 2007; 7: 279–282.
82 Li Y-F, Gauderman WJ, Avol E, et al. Associations of tumor necrosis factor G-308A with childhood asthma and wheezing. Am J Respir Crit Care Med 2006; 173: 970–976.
83 Kerkhof M, Postma DS, Brunekreef B, et al. Toll-like receptor 2 and 4 genes influence susceptibility to adverse effects of traffic-related air pollution on childhood asthma. Thorax 2010; 65: 690–697.
84 Zhao S-T, Wang C-Z. Regulatory T cells and asthma. J Zhejiang Univ Sci B 2018; 19: 663–673.
85 Wei SC, Duffy CR, Allison JP. Fundamental mechanisms of immune checkpoint blockade therapy. Cancer Discov 2018; 8: 1069–1086.
86 Janes PW, Slape CI, Farnsworth RH, et al. EphA3 biology and cancer. Growth Factors 2014; 32: 176–189.
87 Yilmaz O, Yuksel H. Where does current and future pediatric asthma treatment stand? Remodeling and inflammation: bird’s eye view. Pediatr Pulmonol 2016; 51: 1422–1429.
88 Salam MT, Gauderman WJ, McConnell R, et al. Transforming growth factor-β1 C-509T polymorphism, oxidant stress, and early-onset childhood asthma. Am J Respir Crit Care Med 2007; 176: 1192–1199.
89 Saito A, Horie M, Nagase T. TGF-β signaling in lung health and disease. Int J Mol Sci 2018; 19: 2460.
90 Bogard AS, Xu C, Ostrom RS. Human bronchial smooth muscle cells express adenylyl cyclase isoforms 2, 4, and 6 in distinct membrane microdomains. J Pharmacol Exp Ther 2011; 337: 209–217.
91 Nelson MR, Tipney H, Painter JL, et al. The support of human genetic evidence for approved drug indications. Nat Genet 2015; 47: 856–860.