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

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Genetic variants impact the sensitivity to ambient air pollution (AAP) in paediatric patients with asthma. Variants associated with oxidative stress or nuclear factor-κB pathways are linked to AAP sensitivity. https://bit.ly/3wYmLun

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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 (NO_2) [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, NO_2, ozone (O_3), particulate matter (PM) and/or sulphur dioxide (SO_2) [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 NO_2, O_3 and PM.

NO_2 is a combustion byproduct and was initially viewed as mostly an indoor air pollutant [34]. However, since up to 80% of ambient NO_2 is caused by traffic, it has been used as a proxy for TRAP [9, 30]. A limitation of this approach is that while ambient NO_2 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 NO_2 levels as measured outside children's homes have been associated with increased new-onset asthma [30].

O_3 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 O_3 precursors are found in industrial and vehicle exhaust [9, 37]. Increased O_3 has been associated with increased asthma medication usage and morbidity [38–41]. Beyond this, higher mean 4-year O_3 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. PM_{10} and $PM_{2.5}$ 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 PM_{10} and $PM_{2.5}$ 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 O_3 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.

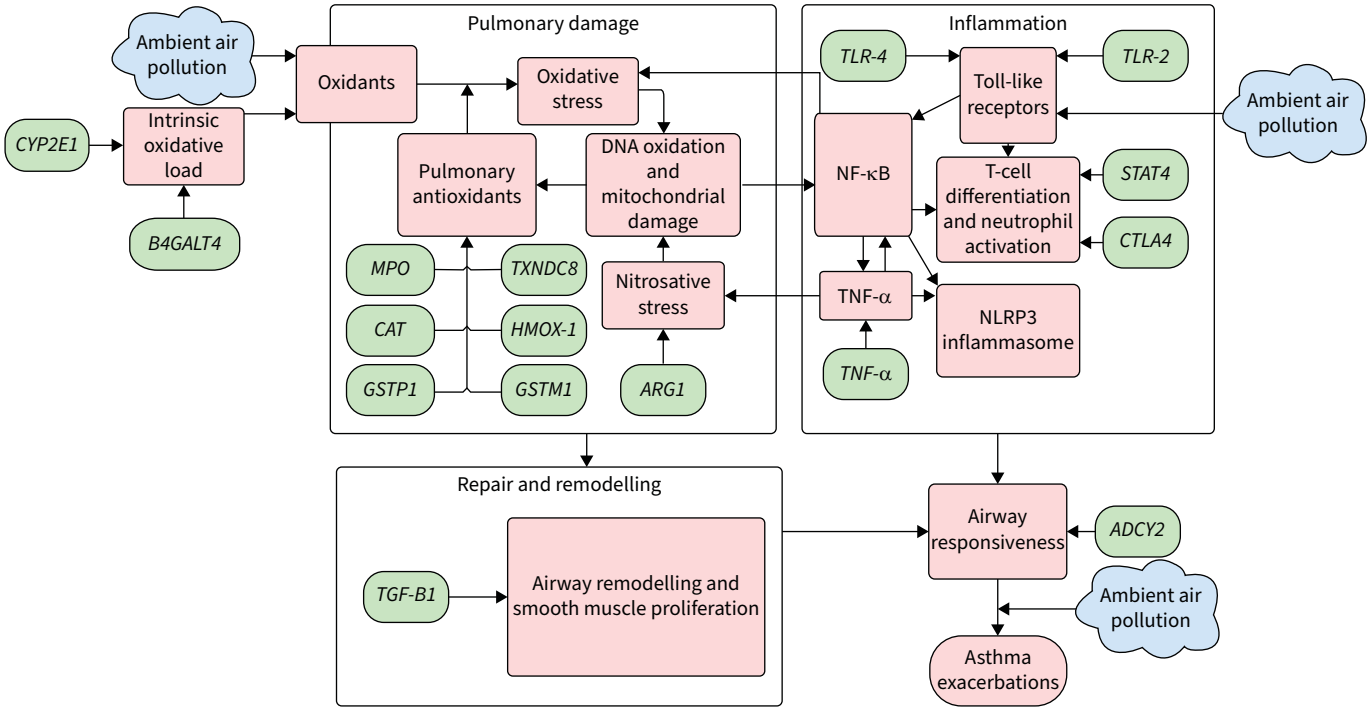

FIGURE 1 Overview of genes and pathways related to pulmonary oxidative stress implicated in ambient air pollution sensitivity. Genes associated with variants that impact ambient air pollution sensitivity are in green ovals and biological processes marked in red rectangles. *ADCY2*: adenylate cyclase 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.

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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 $FEF_{25-75\%}$ after ozone exposure (2.9% decrease, CI 0.6–5.2, per 50 ppb increase in ozone).	Romieu *et al.* [58]
		GSTP1	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).	Lee *et al.* [59]
					Higher risk for childhood asthma for those living in a community with low PM_{10} concentrations (no effect size reported).	Su *et al.* [60]
					Negative association between $PM_{2.5}$ and ozone and childhood asthma (adjusted OR 0.6, CI 0.45–0.82 and 0.74, CI 0.6–0.9, respectively).	Hwang *et al.* [61]
			GSTP1 I/V or V/V at codon 105 (rs947894)	Change in catalytic properties	Increased risk of allergic sensitisation with increasing NO_x exposure (OR 2.4, CI 1.0–5.3 for difference between fifth and 95th percentile exposure).	Melén *et al.* [55]
					Higher risk for childhood asthma for those living in a community with high PM_{10} concentrations (no effect size reported).	Su *et al.* [60]
					Positive association between $PM_{2.5}$ and ozone and childhood asthma (adjusted OR 1.52, CI 1.01–2.27 and 1.19, CI 0.91–1.57, respectively).	Hwang *et al.* [61]
	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).	David *et al.* [63]
	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 NO_2 (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 NO_2 (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 NO_2 exposure in Caucasian children with asthma (no effect size reported).	Ierodiakonou *et al.* [70]

Continued

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TABLE 1 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 NO_2 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 $PM_{2.5}$ exposure (OR 2.0, CI 1.2–3.1 for every interquartile range increase).	Kerkhof *et al.* [83]
		TLR-4	rs2770150 TC rs10759931 GG rs6478317 GG rs10759932 CT or CC rs1927911 TT	Not described	Increased risk for asthma with increasing $PM_{2.5}$ 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 T-lymphocyte-associated protein 4	*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]
	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 NO_2 exposure in Caucasian children with asthma.	Ierodiakonou *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	*ADCY2*	rs6886921 C>T	Not described	Significant interaction with NO_2 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; $FEF_{25-75\%}$: 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 GTSM1 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 O_3 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, NO_x 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, O_3 and $PM_{2.5}$ 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 PM_{10} 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–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 (H_2O_2). Children with variants in both the promotor 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-O_3 community. It was hypothesised that the difference in promotor 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 O_3 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 NO_2 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 O_3 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 $PM_{2.5}$ [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 NO_2 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 NO_2 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 biassing 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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