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Impact of Tobacco Smoke and Nicotine Exposure on Lung Development

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Keywords: Tobacco smoke, lung development, secondhand smoke exposure, thirdhand smoke exposure

Funding: Funded by a Center of Excellence Grant to the American Academy of Pediatrics from the Flight Attendant Medical Research Institute (SM, JMC)

NIH RHL114800A (SM)

Disclosure: None of the authors have any relevant disclosures of interest.

Abstract

Tobacco smoke and nicotine exposure during prenatal and postnatal life can impair lung development, alter the immune response to viral infections and increase the prevalence of wheezing during childhood. The following review examines recent discoveries in the fields of lung development and tobacco/nicotine exposure, emphasizing studies published within the last five years. *In utero* tobacco/nicotine exposure remains common, occurring in approximately 10% of pregnancies within the United States. Exposed neonates are at increased risk for diminished lung function, altered central and peripheral respiratory chemoreception and increased asthma symptoms throughout childhood. Recently, genomic and epigenetic risk factors, such as alterations in DNA methylation, have been identified that may influence the risk for long-term disease. This review examines the impact of prenatal tobacco/ nicotine exposure on lung development with a particular focus on nicotinic acetylcholine receptors. In addition this review examines the role of prenatal and postnatal tobacco smoke/nicotine exposure and its association with augmenting infection risk, skewing the immune response towards a Th2 bias and increasing risk for developing an allergic phenotype and asthma-like symptoms during childhood. Finally this review outlines the respiratory morbidities associated with childhood secondhand smoke/nicotine exposure and examines genetic and epigenetic modifiers that may influence respiratory health in infants and children exposed to *in utero* or postnatal tobacco smoke.

Background

Lung development begins during early embryogenesis and extends into late childhood.^{1;2} Although the majority of postnatal lung growth occurs within the first two years of life, modest alveolar growth continues through the first 15 years of life.³ Exposure to tobacco smoke/nicotine during *in utero* and postnatal life has been shown to disturb lung development, increase susceptibility to lower respiratory tract infections, increase prevalence of wheezing, and exacerbate respiratory symptoms in children with chronic lung diseases.⁴⁻⁸ The goals of this review are to examine the effects of tobacco smoke/nicotine exposure on prenatal and postnatal lung development, assess the relationship between tobacco smoke/nicotine exposure and respiratory morbidities in children, and evaluate the role of genetic and epigenetics on the respiratory phenotypes of children exposed to tobacco smoke/nicotine.

Tobacco smoke contains more than 4000 chemicals, many of which have been associated with deleterious health consequences.⁹ In particular, exposures to nicotine, carbon monoxide, acrolein, and carcinogens including polycyclic aromatic hydrocarbons, aromatic amines, and tobacco specific N-nitrosamines can be detrimental, especially during fetal life and periods of rapid postnatal organ growth, including the lungs and brain.^{4;10} Nicotine, the primary addictive substance in tobacco smoke, has been shown to cross the placenta *in utero* as evidenced by high levels of cotinine, a nicotine metabolite, in the amniotic fluid of pregnant smokers and in blood spots from neonates exposed *in utero*.^{11;12} Nicotine has also been shown to be concentrated in the breast milk of mothers who smoke.^{13;14} In addition to indirect maternal exposure, children can also be exposed to secondhand tobacco smoke and/or thirdhand tobacco smoke during postnatal life. Secondhand smoke (SHS) is a mixture of mainstream smoke exhaled from the smoker and sidestream smoke that enters the environment from the burning cigarette. More recently,

thirdhand smoke exposure has been recognized as a source of tobacco smoke/nicotine exposure particularly in indoor environments. Thirdhand smoke exposure occurs when constituents of tobacco smoke deposit on surfaces. These chemicals may attach to dust, be re-emitted into the air, and/or react with other chemicals in the environment.¹⁵ Due to oxidation and breakdown processes, thirdhand smoke may have enhanced toxicity compared to mainstream smoke. Furthermore, infants and young children may be at highest risk for toxicity from secondhand and thirdhand smoke exposure since they tend to spend more time indoors, have faster respiratory rates, a greater relative body surface area and more hand/oral behaviors than older children.

Tobacco smoke exposure during prenatal and postnatal lung development is associated with an increase in respiratory morbidities during childhood. Tobacco smoke exposure can adversely affect lung development, cause small airway dysfunction and wheeze, and increase the frequency of upper and lower respiratory tract illnesses.¹⁶⁻¹⁸ It is unclear if links exist between tobacco smoke exposure during childhood and increased risk of chronic obstructive pulmonary disease (COPD) during adult life. However, recently Chan and colleagues found an association between childhood pneumonia, and diminished adult lung function.¹⁹ This finding suggests that the risk of COPD in adult life may be influenced by SHS during childhood, since children exposed to SHS are more likely to have a history of lower respiratory tract infections.

The detrimental effects of childhood tobacco smoke exposure on respiratory health may be increased by other confounding factors including psycho-social stress, lower socioeconomic status, underlying chronic lung disease, genetic polymorphisms, and exposure to environmental pollutants.²⁰ SHS exposure may also impair the effectiveness of corticosteroids used for the treatment of small airway disease.²¹ In addition, adverse health effects caused by SHS may be

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augmented in children with a genetic susceptibility to asthma while exposure to tobacco smoke during *in utero* life may cause epigenetic changes in fetal lung.²²

Prevalence of tobacco smoke exposure during *in utero* and early childhood postnatal life

Prenatal exposure of children to tobacco smoke remains high in the United States and worldwide, and caregiver questionnaires may underrepresent the actual number of children exposed to secondhand smoke.⁶ In the United States, an estimated 400,000 newborns are exposed every year to tobacco smoke during pregnancy, with 10.7% of pregnant mothers smoking during the last three months of pregnancy.²³ The highest prevalence of smoking has been reported in women between 20 and 24 years of age (17.6%).²³ Although multiple tobacco toxins likely affect the fetus, nicotine is the best studied component of tobacco smoke. Nicotine readily crosses the placenta and can cause significant *in utero* exposure to the fetus. Indeed, cotinine levels in neonates exposed to *in utero* tobacco smoke have been reported to be similar to that of active smoking adults.^{24;25} Analysis from the Pregnancy Risk Assessment Monitoring System for 2001/2002 revealed that maternal smoking was associated with a longer length of stay in the neonatal intensive care unit, with an estimated smoking attributable cost of 122 million dollars annually in the United States.²⁶

Postnatal exposure of children to SHS continues to be widespread. Oberg and colleagues, using data from 192 countries in 2004 reported that 40% of children worldwide were exposed to secondhand smoke.²⁷ They also estimated that 5,939,000 lower respiratory infections in children less than 5 years of age were due to secondhand smoke exposure and that 28% of the approximately 379,000 deaths annually due to SHS occur in children. Despite these and numerous other studies which have shown links between tobacco smoke exposure and increased

morbidity and mortality in children, prenatal and postnatal exposure of children to tobacco/nicotine products remains unfortunately common.

Normal lung development

Human lung development spans both prenatal and postnatal life,¹ and can be adversely affected by exposure to tobacco smoke/nicotine during both of these periods. During normal embryogenesis (0-6 weeks gestation) lung buds develop from the ventral foregut by week 4 of gestation with lobar and subsegmental branching with concomitant vascular growth taking place by week 6. During the pseudoglandular stage (7-16 weeks gestation) the conducting airways and pre-acinar pulmonary vessels form completely. Airway cartilage, smooth muscle, and mucous glands appear, and epithelial cell differentiation progresses from proximal to distal sites. The canicular stage of lung development (16-24 weeks gestation) signal the earliest development of terminal respiratory units. This stage of lung development is characterized by the formation of type I and type II epithelial cells, capillary proliferation, thinning of pulmonary mesenchyme, and closer contact of capillary vessels to alveolar epithelium. The saccular stage of lung development (weeks 24-40) is characterized by increasing surface area of the gas exchanging units, formation of true alveoli and maturation of the surfactant in the type II epithelial cells. The majority of postnatal alveolar growth occurs during the first two years of life with slower growth into adolescence.^{3;28}

In animal models, tobacco smoke/nicotine exposure during *in utero* and/or early postnatal life has been shown to alter expression of genes involved in cell proliferation, Th1/Th2 immune balance, fibrosis and viral immune responses (Table 1).

Nicotine, nicotinic acetylcholine receptors and lung development

Nicotinic acetylcholine receptors (nAChRs) are ligand gated ion channels that enable flow of cations through the channel when bound by acetylcholine and other nAChR agonists, including nicotine. These receptors consist of five transmembrane subunits that form a central ion pore.²⁹ Heteromeric nAChRs possess alpha and beta subunits, while alpha-7 nicotinic receptors are homopentamer subunits. Human bronchial and endothelial cells have been reported to express $\alpha 3/\beta 5$ and $\alpha 7$ nicotinic acetylcholine receptors.³⁰ nAChRs have been shown to be expressed in the brain and lung during early fetal life, thus representing a potential mechanism for the effects of nicotine on development.

In utero nicotine exposure has been reported to disrupt normal developmental processes in the brain and lungs.^{31;32} The effects of nicotine may occur very early in lung development. For example, Ben-Yehudah and colleagues have shown that non-human primate embryonic stem cells (nhESCs) express nAChRs.³³ In their study, exposure of nicotine caused downregulation of n-Myc expression and differentiation of nhESCs into fibroblasts demonstrating that exposure to nicotine during embryogenesis can affect embryonic stem cell differentiation. The effects of nicotine may also occur later in fetal development as seen in animal studies. Sekhon and colleagues implanted nicotine pumps in pregnant rhesus monkeys and found reduced total body weight and alveolar hypoplasia in the offspring. They also found that nicotine increased expression of α -7 receptors in airway cartilage and vessels of fetal lung, while other nicotinic receptor subtypes although expressed in fetal lungs, were not upregulated by nicotine.³⁴ Prenatal nicotine exposure in mice has been shown to lead to diminished forced expiratory flows in adulthood, but interestingly not in α -7 nAChR knockout mice.³⁵ Other rodent studies have found similar findings with impaired lung growth and alveolar development.^{36;37} A recent study of lung

tissue from fetal and infant sudden death cases found a higher incidence of α -7 nAChR immunostaining in lung associated with maternal smoking and maternal smoking was linked to lung hypoplasia and hypoplasia of specific brainstem regions.³⁸ In addition to nicotine, other components of tobacco smoke may be detrimental to the developing lung. Further research is needed to delineate the relative contributions of tobacco constituents to adverse lung development.

Vitamin C have been tested as a therapy to mitigate the effects of *in utero* smoke exposure. Proskocil and colleagues reported that Vitamin C when given to pregnant rhesus monkeys, improved lung function in nicotine-exposed offspring.³⁹ A recent randomized placebo controlled study found that the offspring of smoking mothers who received Vitamin C during *in utero* life had better air flow and less wheezing through the first year of life compared to untreated mothers who smoked during pregnancy.⁴⁰ The mechanism underlying the potential protective effect of Vitamin C against *in utero* smoke exposure is not clear.

Maternal smoking and Sudden Infant Death Syndrome

Tobacco smoke exposure has been linked to an increased risk of sudden infant death syndrome (SIDS). A recent study found that maternal smoking was reported in 38.6% of infants dying of SIDS.⁴¹ Mitchell and Milerad, previously reported that the pooled relative risk of SIDS associated with maternal smoking was 2.86. Furthermore if a causal association was assumed, one-third of SIDS deaths may have been avoided if the infant had not been exposed to smoke in utero.⁴²

The mechanisms underlying this link between prenatal tobacco smoke exposure and SIDS have not been clearly elucidated. Rodent studies have indicated that nicotine exposure during prenatal life may alter central and peripheral respiratory chemoreception.^{43;44} A study in

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neonatal rats found that pups exposed to prenatal cigarette smoke had prolonged gasping following a hyperthermic/hypoxic exposure.⁴⁵ Another study reported that neonatal mice exposed to *in utero* nicotine had decreased ventilation and a reduced response to hypercarbia when exposed to 100% N₂ for 20 seconds and 10% CO₂ for 20 minutes.⁴⁶

***In utero* and postnatal SHS exposure and its impact on lung function in children**

Both *in utero* and postnatal SHS exposure have been associated with increased respiratory symptoms and/or reduced lung function in exposed children. It has been difficult however, to parse out the effects of *in utero* exposure versus postnatal exposure, particularly as many children are exposed to both. In 3 year old children within the Generation R cohort there was an increased risk of wheezing with any prenatal or postnatal tobacco smoke exposure, but at four years of age, those with postnatal exposure alone did not have increased wheezing.^{5;47} Hayatbakhsh and colleagues also found that adult males with prior *in utero* smoke exposure had significant reductions in FEV₁ and FEF_{25–75}.⁴⁸ Another study reported that Chinese school-aged children exposed to SHS had increased cough and reduced lung function compared to unexposed children.⁴⁹ A Finnish study that examined young children with a history of multi-trigger wheezing found that children of mothers who smoked, validated by cotinine levels, were more likely to have increased airway resistance and higher FENO levels than non-exposed children.¹⁷ Aberrant growth and inflammation of the small airways from tobacco exposure during *in utero*/early postnatal life may in part account for the observed symptoms and outcomes in these studies. Wongtrakool and colleagues, found an increase in α -7 nAChRs and stimulated lung branching in nicotine treated embryonic murine lung explants suggesting that *in utero*

nicotine exposure may lead to dysnaptic airway growth and reduced lung function in exposed offspring.⁵⁰

***In utero* and SHS exposure and its impact on respiratory infections**

Respiratory tract infections represent a major source of morbidity in infancy and early childhood.⁵¹ These infections have developmental consequences as they are increasingly recognized as a source of subsequent respiratory disease sequelae and diminished adult lung function.^{19;52} Since the 1980s, studies have demonstrated increased risk for lower respiratory tract infections in infants exposed to maternal smoke.^{53;54} The risk for subsequent infection however is not uniform between prenatal and postnatal exposure. Prenatal maternal smoking may carry a higher risk of subsequent infection, suggesting a developmental mechanism for the increased vulnerability,^{55;56} but the underlying mechanisms that account for this increased infection risk are not fully understood. Limited animal and human data suggest that tobacco products alter both the innate and adaptive immune response.

Effect of tobacco smoke exposure during *in utero* and early postnatal life on pulmonary immune responses

Coordinated activation of the innate and adaptive immune systems is critical to effective clearance of pathogens and resolution of infections. The immune system in fetal life faces the added challenge of preventing potentially lethal inflammatory responses to maternal alloantigens. Consequently, fetal T helper (Th) cells exhibit a predominately anti-inflammatory Th2 response.⁵⁷ After birth the immune response normally shifts towards a Th1 immune response. In animal models exposure to *in utero* and early postnatal tobacco smoke has been associated with a Th1/Th2 imbalance, and increased susceptibility to lower tract respiratory

infections.⁵⁸ Human studies have found altered cytokine profiles in tobacco-exposed neonates. Prenatal exposure to tobacco smoke can cause decreased levels of cord blood interferon γ and continued environmental tobacco smoke exposure has been shown to suppress interferon γ levels through 11 years of age.^{56;59} Alteration in interferon γ production may be particularly relevant for infection risk as Sumino *et al.* recently correlated decreased monocyte interferon production at birth with increased risk of early life infection.⁶⁰ In addition to decreasing interferon γ , *in utero* smoke increases the levels of cytokines associated with Th2 immune profiles. Noakes *et al.* found that cord blood from neonates exposed to *in utero* smoke had increased levels of IL-13 after antigen challenge.⁶¹ However there is sparse human data on immune cell profiles and function to support the cytokine studies. Indeed in a recent study comparing neonates born to smoking and non-smoking mothers, Almanzar *et al.* found no difference in lymphocyte subsets between the two groups.⁶²

Animal models, however, support immune cell phenotypic changes after *in utero* smoke/nicotine exposure (**Figure 1**). Neonatal mice exposed to *in utero* nicotine had enhanced M2 phenotype in their alveolar macrophages.⁶³ Similarly, in a primate model, Wang *et al.* demonstrated decreased Th1 cytokine production after *in utero* and postnatal smoke exposure, which correlated with decreased recruitment of Th1 immune cells to the lung parenchyma.⁶⁴ In addition, decreased expression of interferon-inducible GTPases and type 1 interferon response pathway genes induced by viral double-stranded RNA were found in the lungs of neonatal mice exposed to secondhand smoke.⁶⁵

Along with modifying infection risk, the secondhand smoke skewed immune response towards a Th2 bias may result in an allergic phenotype, small airway inflammation, and asthma-like symptoms. In animal models exposure to *in utero* and early postnatal tobacco smoke has

been associated with a Th1/Th2 imbalance, and an increased susceptibility to airway reactivity.⁵⁸ Wang and colleagues examined the Th1/Th2 pulmonary responses in lungs of infant monkeys exposed to *in utero* and early postnatal tobacco smoke and found decreased expression of upstream and downstream interferon- γ pathway genes that regulate interferon- γ .⁶⁴

Thus, smoke exposure may render neonates vulnerable to infection and promote development of allergic disease by changing the activation profile of immune cells; however, further research is needed both to establish the changes in cellular immunity in humans and also to define the molecular pathways underlying these changes.

Epigenetic effects of tobacco smoke exposure

Although the deleterious clinical consequences of early life tobacco exposure have been well established over the past several decades, the molecular mechanisms underpinning these changes remain incompletely understood. In recent years, epigenetic interactions with tobacco smoke have emerged as potentially intriguing mechanisms for postnatal lung disease. Epigenetic modification of gene expression and phenotype plays an important role in fetal development, furthermore the epigenome that develops *in utero* and in early life persists throughout life and is heritable.⁶⁶

Tobacco-related epigenetic changes, particularly DNA methylation, were first noted in the context of cancer development and progression.^{67;68} Subsequently epigenetic differences in global DNA methylation have been found between smokers and non-smokers.^{69;70} Due to difficulties in sampling, the majority of epigenetic studies of fetal methylation patterns in humans have been performed in cord blood and placenta. Cord blood studies have implicated smoking-induced methylation changes in multiple specific genes including *CYP1A1*, *GFI*,

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FOXP3, and *AHRR*,⁷¹⁻⁷³ which play a role in detoxification and immune regulation and therefore may contribute to childhood lung disease. Among the best understood and most consistently recovered changes in methylation patterns involves the *AHRR* gene, which encodes for the aryl hydrocarbon receptor repressor, a feedback inhibitor which has been suggested to attenuate effects of tobacco smoke toxins.⁷⁴ Studies of human cord blood have shown decreased methylation in *AHRR* loci in neonates similar to immune cell methylation patterns in adult smokers. These neonatal changes correlated with maternal levels of cotinine.⁷³ These epigenetic changes in *AHRR* methylation persist well into post-natal life. Novakovic *et al.* recently demonstrated persistent *AHRR* hypomethylation in blood monocytes at 1 year of age,⁷⁵ and a separate longitudinal study detected persistent changes at 7 years of age.^{71;76} Changes in *AHRR* methylation may contribute to altered detoxification function and subsequent disease development. Placental studies also have demonstrated changes in global and gene specific methylation.

Although these findings are highly suggestive, placental and cord blood studies do have limitations. Significant variability in methylation patterns between cord blood, placental and saliva samples suggests tissue specific epigenetic patterns,⁷⁷ which may lead to differing functional consequences.⁷⁸ Currently, knowledge of tissue specific-epigenetic effects of tobacco smoke exposure remains limited. One of the first papers looking at the effects of *in utero* smoke exposure on fetal lung-specific methylation found changes in methylation of *DPP10*, a gene associated with asthma.²² Ultimately, further work is needed to correlate cord blood, placental and tissue specific epigenetics with functional measures of lung disease.

Intriguingly, the epigenetic effects of tobacco smoke may extend beyond direct effects on gene expression to transgenerational changes in lung development. In a well-established animal

model of *in utero* nicotine exposure, Rehan *et al.* demonstrated increased airway resistance and contractility proteins in rats that were one generation (F2) removed from *in utero* nicotine exposure, which was supported by tissue specific epigenetic differences.⁷⁹ Human data evaluating the transgenerational effects of tobacco smoke on lung function has shown differing results, possibly due to unmeasured confounders such as socioeconomic status, distinct environmental exposures, or ethnicity. A grandmaternal effect on asthma risk was first suggested by the Children's Health Study in 2005.⁸⁰ In this nested case control study, grandmaternal smoking was associated with a 2.1 OR risk of developing childhood asthma; however, this effect was unable to be replicated using data from the Avon Longitudinal Study of Parents and Children.⁸¹ The Norwegian Mother and Child Cohort Study (MoBa) also found a strong association between grandmaternal smoking during pregnancy and childhood asthma.⁸²

Ultimately, tobacco smoke-related modification of the epigenome appears to have longitudinal effects on DNA methylation patterns and may even alter transgenerational risk for the development of lung disease. There is a need for future research to better define the functional consequences of epigenetic modification, the heritable consequences of *in utero* smoke exposure and the role for therapies that target smoke's epigenetic modifications.

Gene-environment interactions and tobacco smoke exposure

Like with epigenetics, the interplay between genes and environmental exposures is becoming increasingly important to our understanding of the development of complex lung diseases. Both chromosomal linkage studies and analysis of specific polymorphisms have highlighted the role of genetics in determining susceptibility to *in utero* smoke exposure. Much of the research has focused on the association between genes, smoke exposure and subsequent

asthma; however, few studies have looked globally at the genome. The first genome-wide interaction study of early life tobacco exposure and asthma was published in 2014 and identified novel polymorphisms on chromosome 6 and 18.⁸³ Linkage studies have also implicated chromosomal regions 1p, 1q, 5q, and 17p as modifying asthma risk after early life smoke exposure

Specific candidate genes have emerged from the growing body of research on asthma genetics. Research on candidate genes has largely focused on xenobiotic detoxification and antioxidant pathways, including the *GST* family. Encoding for the glutathione S-transferase enzymes, *GSTs* play a role in metabolizing tobacco products and reactive oxygen species. Deletions of these genes are common, with 50% and 25% of Caucasians being homozygous *GSTM1* null and *GSTT1* null respectively. Polymorphisms have been implicated in asthma pathogenesis.⁸⁴ The first study to associate *in utero* smoke and GST enzymes found that *GSTM1* null children had increased risk of both diagnosed asthma and wheezing after *in utero* smoke exposure compared to individuals with functional copies.⁸⁵ Subsequent research has demonstrated physiologic changes, including increased airway resistance and decreased lung function after *in utero* smoke exposure in children with *GSTT1* and *GSTP1* alleles.^{84;86} Wu and colleagues reported in a population-based birth cohort that maternal smoking increased the risk of wheezing in children who had a *GSTP1 AA* genotype, however the effect of maternal smoking on wheeze diminished with age.⁸⁷ Additional candidate genes, *CYP1A1*, *ADAMS33* and *TNF*, have been suggested to modify the risk of subsequent asthma.^{83;88;89} Further genome wide association studies and clearer understanding of molecular pathways are needed to validate the role of identified candidate genes in the interaction between *in utero* smoke and lung development.

Electronic cigarettes and lung development

With the increased popularity of electronic nicotine delivery systems (ENDS) among people of child bearing age, it is unknown what effect nicotine containing Electronic cigarette (E-cigarette) aerosols will have on the lung function and respiratory health of children exposed during *in utero* and early postnatal life. One study reported that non-E-cigarette users exposed to E-cigarette aerosols in the environment have similar cotinine levels as those exposed to secondhand smoke from tobacco containing cigarettes.⁹⁰ Recent animal studies in mice have shown an adverse effect on E-cigarette aerosols on postnatal lung development and the innate immune system.^{91;92} E-cigarette juices come in a variety flavors with and without nicotine. Lerner and colleagues reported increased levels of the pro-inflammatory cytokines, IL-6 and IL-8 in human epithelial cells and fibroblasts exposed to nicotine and non-nicotine flavored E-cigarette juices.⁹³ Furthermore they reported that the lungs of mice exposed to nicotine containing E-cigarette aerosols had decreased total glutathione. Taken together these findings indicate that exposure to E-cigarette aerosols may increase oxidative stress and inflammation in the lung.⁹³ Although data are limited regarding the pulmonary effects of E-cigarette aerosols on fetal development, the nicotine component of these aerosols may have similar developmental consequences to other forms of nicotine delivery. A recent study from the United Kingdom reported that mothers given nicotine replacement therapy (NRT) during their first trimester or a month prior to conception had a significant increase in respiratory anomalies but not any other organ specific system.⁹⁴ However ENDS have the capacity to deliver a high level burst of nicotine. This in turn may cause different physiologic or pathologic changes in the developing lung compared to NRT.

Gaps in knowledge and future directions

Studies that continue to examine the effects of tobacco exposure during *in utero* life and throughout childhood are needed to fully understand the long-term effects of tobacco smoke exposures on adult lung function. Additionally, studies that elucidate the role of epigenetic changes and genetic polymorphisms on respiratory disease phenotype in children exposed to secondhand smoke would help identify subgroups of individuals who may be at highest risk for respiratory morbidities and the development of COPD. Finally studies are needed that examine the short and long-term effects of E-cigarette aerosols on lung development and childhood respiratory health.

Figure Legend

Figure 1. Schematic of phenotypic immune cell changes caused by tobacco smoke or nicotine exposure. Macrophages (M) exposed to tobacco smoke/nicotine are skewed towards a M2 phenotype whereas T helper (Th) cells exposed to tobacco smoke/nicotine are skewed towards a Th2 phenotype. This preferential skewing caused by tobacco smoke/nicotine exposure results in increased levels of IL13 and decreased levels of interferon γ .

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Table 1. Effects of tobacco smoke/nicotine exposure on gene expression during development

Changes in gene/receptor expression	Effects of tobacco smoke/nicotine exposure	Reference
↓ nMyc expression	Nicotine caused fibroblast differentiation in non-human embryonic stem cells	Ben-Yehudah et al. ³³
↑ α-7 nicotinic acetylcholine receptors (nAChRs)	Prenatal nicotine exposure caused ↑ α-7 nAChRs in airway, cartilage, vessels in rhesus monkeys	Sekhon et al. ³⁴
↓ IFNγ (Interferon gamma) ↓ Tbet (Th1 specific transcription factor) ↑ Th2 signaling pathway (GATA3/Lck/Erk/STAT6)	Prenatal smoke exposure caused Th1/Th2 changes in mice after <i>Aspergillus</i> sensitization	Singh et al. ⁵⁸
↑ CTGF (connective tissue growth factor)	↑ lung fibrosis in rats exposed to prenatal/postnatal nicotine and hyperoxia	Huang et al. ⁹⁴
↓ Interferon-inducible GTPases and genes activated by double-stranded viral RNA	↓ expression of innate immunity response genes in neonatal mouse lung exposed to postnatal tobacco smoke	McGrath-Morrow et al. ⁶⁵

