Early in Life Epi-Genetic Events and the susceptibility to Chronic Inflammatory Lung Diseases

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Abstract
Chronic inflammatory lung diseases (CILD) are increasing worldwide and represent a major issue for daily life performance and public health expenditure. The two major CILD are asthma and chronic obstructive pulmonary disease (COPD). The World Health Organization (WHO) estimated that 280 million people currently suffer from asthma and 40% of all children experience asthma symptoms. COPD affected 65 million people with a death rate of 3 million/year in 2005 (WHO). An increasing number of studies indicate that the likelihood to develop asthma or COPD is linked to inheritable susceptibility factors. However, despite of large genetic cohorts, no specific candidate genes had been identified. Instead, epi-genetic events induced by known CILD risk factors can mimic inheritance. The mechanism how epi-genetic events can be transmitted over generations is unknown. This review aims to provide an overview on the current knowledge on epi-genetic events that can be induced by known CILD risk factors and which contribute to CILD during adulthood. The available data points towards epi-genetic mechanisms rather than the modification of the DNA.

Future studies have to investigate the mechanisms which make epi-genetic events “inheritable”. Preventive strategies have to be developed and pregnant women have to be informed about the risk factors which pre-dispose their children to suffer from lifelong malfunction of the lung. Strategies to reduce the burden of CILD can only be achieved by projects that link basic, clinical and epidemiological research. However, as long as the public is not aware of the problem, such studies will have difficulties to obtain funding.

Introduction
Chronic inflammatory lung diseases (CILD) include asthma, chronic obstructive pulmonary disease (COPD), lung fibrosis, and other dust induced lung diseases, which are increasing worldwide. Unfortunately, the available therapies only reduce the symptoms but do not provide cure for any CILD. Beside COPD which is clearly related to cigarette smoking or exposure to fire smoke, the increase of other CILD is not well understood.

An increasing number of studies indicate that the likelihood to develop asthma or COPD is linked to inheritable susceptibility factors. However, despite of large genetic cohorts, no specific candidate genes had been identified. Instead, epi-genetic events induced by known CILD risk factors can mimic inheritance. The mechanism how epi-genetic events can be transmitted over generations is unknown. This review aims to provide an overview on the current knowledge on epi-genetic events that can be induced by known CILD risk factors and which contribute to CILD during adulthood. The available data points towards epi-genetic mechanisms rather than the modification of the DNA.

Future studies have to investigate the mechanisms which make epi-genetic events “inheritable”. Preventive strategies have to be developed and pregnant women have to be informed about the risk factors which pre-dispose their children to suffer from lifelong malfunction of the lung. Strategies to reduce the burden of CILD can only be achieved by projects that link basic, clinical and epidemiological research. However, as long as the public is not aware of the problem, such studies will have difficulties to obtain funding.
The quest for such a mechanism is further complicated since several studies over three generations suggested that the susceptibility to develop COPD or CILD may be determined by cigarette smoking of grandmothers and mothers [7,8]. Indirect evidence of epigenetic events being important contributors to asthma susceptibility comes from studies of moth child cohorts. The exposure of mothers to phthalates during pregnancy increased the risk of developing asthma, wheeze, and atopic diseases later in life significantly [11]. In maternal nutrition during pregnancy increased the risk of developing in the next two generations; however, the mechanism remains unclear [9]. In a polish study it was shown that low zinc levels correlated with an increased risk of asthma [10]. In a meta-analysis it was shown that maternal nutrition during pregnancy increased the risk of developing asthma, wheeze, and atopic diseases later in life significantly [11]. In addition, risk factors for CILD development could be mediated by breast feeding [12]. The only known mechanisms to result a lasting modification of DNA methylation [18,19]. Interestingly, reported that the methylation pattern of the gene encoding AHRR that were observed in adult asthma patients [16,17]. Another study methylation in children exposed to CILD risk factors are the same unknown proteins or other regulatory factors such as micro RNAs.

DNA methylation was studied in 527 aged 5-12 years with a history of prenatal smoking of their mothers [14]. This study investigated the methylation status of 20, 578 DNA sequences and observed that specifically Cp G loci were affected. However, most of the identified genes which were linked to such a Cp G loci have no known function and their effect on development of CILD remains to be studied. In a similar study, DNA methylation caused by prenatal exposure to cigarette smoke was studied in cord blood from 572 children who showed signs of respiratory symptoms at the age of 3-5 years [15]. Using a DNA methylation array, the researchers reported at least 10 folds increase of tobacco induced genes specific methylation patterns for 26 different genes. Several of the identified genes which are modified by the tobacco smoke regulate innate immunity such as Aryl Hydrocarbon Receptor Repressor (AHRR) and cytochrome P450 (CYP); unfortunately, about 50% of these genes encode for yet unknown proteins or other regulatory factors such as micro RNAs. It remains to be investigated if these genome wide patterns of DNA methylation in children exposed to CILD risk factors are the same that were observed in adult asthma patients [16,17]. Another study reported that the methylation pattern of the gene encoding AHRH which was stimulated by prenatal smoke exposure persisted during early childhood indicating that environmental factors can provoke a lasting modification of DNA methylation [18,19]. Interestingly, there might be a link between epigenetic mechanisms and the loss of childhood asthma during puberty. In a cohort of 245 females, blood samples were collected at the age of 10 and 18 years and a comparison of DNA methylation was done and showed that the methylation of genes encoding for Th2 cytokines and regulators were negatively correlated with asthma [20]. There is also the possibility that methylation dependent inactivation of one X-chromosome is inherited through the maternal line, but the mechanism(s) by which methylation is stabilized is unknown [18].

In order to be inheritable, these modifications would be most effective if they occur in mitochondria genes, which are only forwarded to the next generation through the oocytes and therefore, only through the maternal line. Malfunction and over activity of mitochondria has been reported in asthma and were related to airway smooth muscle cell hyperplasia and increase secretion of pro-inflammatory cytokines [21]. Epigenetic deregulation of mitochondria genes has been associated with aging [22] and for their role in resetting the gene regulatory network during embryogenesis [23]. Furthermore, mutations or epigenetic modification of mitochondrial genes have been linked to the regulation of nuclear genes, which regulate inflammation, proliferation, and cell differentiation [24]. There is also an indication that micro RNAs can affect mitochondria gene activity, however, these studies have only recently been started and need further investigation [25].

**CILD in Animal Studies**

The risk to develop CILD through an epigenetic mechanism to be activated during very small time windows in embryonal development was implied twenty years ago in a mouse model [26]. The increased susceptibility to develop CILD was maintained over three generations and was linked to Hypo-Methylation of two CILD relevant cytokines, IL-4 and IFN-γ. Based on studies in the context of tumor genesis we know that hypo-methylation of genes is associated with increased transcription of pro-inflammatory genes, while only one study suggested its role in the pathogenesis of asthma [27]. In a rat model of nicotine induced methylation patterns the transgenerational transmission of asthma susceptibility was reported to be transmitted over three generations, while its irreversible nature remained unclear. More surprisingly, abnormal lung function and increased expression of fibro nectin was linked with an organ specific increased DNA methylation and affected only male offspring. The study also reported increased methylation of his tones H3m while being reduced in histone H4 [28]. In mice, DNA methylation was sensitive to stimulating environmental factors during two stages: i) directly after fertilization affecting all cells of the developing embryo; and ii) when germ line forming cells developed in the embryo; the latter presents a mechanism that leads to lasting, inheritable conditions which will only be effective in the third generation [29]. Cigarette smoke exposure of pregnant mice distinctly the expression growth factor and Wnt-receptors, as well as of the corresponding signaling protein in their offsprings [30]. Modified Wnt-signaling contributes to tissue remodeling properties of human asthmatic airway smooth muscle cells, where Wnt 5a/b and the corresponding receptors affected TGF-β1-induced extracellular matrix synthesis [31]. The mechanism how such epi-genetic modifications become organ specifically inherited through the maternal line remains open.

Studies in non-human primates showed that several CILD pathologies can be induced by exposure to allergens, cigarette smoke, or increased oxygen radicals during pregnancy and early infancy [32-35]. Not only the distribution of lung and airway epithelial cells, but also their function was lastingly modified by the relative short
Yammine et al. [54] provided indirect evidence of postnatal maturation of the fetal lung [53]. On the contrary, a study by treatment on mothers, who had a risk of preterm delivery, accelerated a total of 1,557 women and 1,661 infants showed that glucocorticoid infant at birth [52]. A meta-analysis including 12 clinical trials with system is activated by oxygen contained in the inhaled air of the lung at birth [51]. Both lung maturation and the pulmonary surfactant system which is activated by the lung inflation with air at birth [51]. The link of reduced lung function in premature-children is incomplete at birth and the lung cannot reach maturity [48,49]. These findings indicated that lung maturation in preterm children is at complete and the lung cannot reach maturity after birth [48,49]. The link of reduced lung function in premature-born children later in life was confirmed by a meta-analysis including 25,000 children [50]. Lung maturation is limited by the pulmonary surfactant system which is activated by the lung inflation with air at birth [51]. Both lung maturation and the pulmonary surfactant system is activated by oxygen contained in the inhaled air of the infant at birth [52]. A meta-analysis including 12 clinical trials with a total of 1,557 women and 1,661 infants showed that glucocorticoid treatment on mothers, who had a risk of preterm delivery, accelerated the maturation of the fetal lung [53]. On the contrary, a study by Yammine et al. [54] provided indirect proof of postnatal maturation of alveoli. However, these studies were based on indirect measures such as lung function and exhaled gas composition, thus direct evidence for reduced lung structure maturation is missing.

The inheritance of the asthma and COPD has been described and discussed for long, but none of the proposed genetic condition had ever been proven to be essential for the initiation of these diseases [5,6]. There is epidemiologic evidence that the development of COPD later in life is linked to modified gene activity which is somehow fixed during embryogenesis. The nature of the underlying mechanism leading to CILD on the level of cell biology is not clear and seems to involve less well known epigenetic mechanisms that occur during embryogenesis and are therefore also effective when a child is born pre-term.

Among all animal models described above, the non-human primate rhesus monkey model represents the closest similarity of human lung development, structure, and function for studying COPD and asthma pathogenesis [32]. Several of these studies provided first evidence that lung development is affected by exposure of mothers to environmental CILD risk factors. Other animal models have shown that oxygen determines the differentiation of the lung epithelial cells surfactant proteins at birth, which are regulated through C/EBP-α and the hypoxia inducible factor 1α [55- 57]. Furthermore, lung maturation was controlled by the length of gestation and involved two cell differentiation regulating transcription factors, C/EBP-α and cAMP Response Element Binding protein (CREB) [49].

C/EBP-α, is a central regulatory factor for lung development and cell differentiation [48, 58-60] and in response to hormones during lung maturation [48,61]. The expression control of C/EBP-α is regulated most probably through epigenetic mechanisms which may involve mRNA binding proteins such as calreticulin affecting gene translation and activation [62,63]. In regard to asthma we reported the lack of C/EBP-α specifically in airway smooth muscle cells [64], which resulted from missing translation, while mRNA was present, and which was inducible in vitro by house dust mite allergen exposure [65]. C/EBP-α is an important regulator of cell differentiation in other cell types, but also plays an important role in the response of airway mesenchymal cells to glucocorticoids and long acting β2-agonists which are the main therapeutic drugs prescribed for treatment of asthma and COPD [64,66]. Due to the fact that all C/EBP-iso forms competitively bind to the same DNA promoter sequence and have opposing effects [58] the lack of a negative regulatory element such as C/EBP-α will result in increased proliferation and inflammatory cytokine release without the need to increase the synthesis or activation of other C/EBP-iso forms.

In COPD, C/EBP-β is more central for cell function and response to environmental CILD risk factors [67]. In this context it is interesting that cigarette smoke modifies the expression of C/EBP-β in epithelial cells differently from other cell types [68]. Furthermore, other C/EBP-iso forms may also affect bronchial epithelial cell development, function and immune system activation probably through their competitive binding to identical DNA promoter sequences [69,70].

On the level of anti-oxidants, reduced glutathione levels were reported in preterm born children’s lung fluids, and were considered as an indicator for development of bronchopulmonary dysplasia reducing lung function [71,72]. In isolated human asthma airway smooth muscle cells glutathionylation of IκB reduced TNF-α induced inflammatory response [73]. Importantly, the reduced activity of

**Figure 2:** Epi-genetic events that were transmitted from mother to child in animal studies. There is no direct evidence that the methylation/de-methylation of histones is the cause of de-regulated proteins, except for IL-4 and IFN-γ.
glutathione transferase can be induced by maternal smoking during pregnancy and exposure to environmental cigarette smoke during early childhood, and thus may aggravate the effect of reduced glutathione [74]. Furthermore, in smoking pregnant women the loss of glutathione S-transferase Mu 1 was related to reduce lung function of their children later in life [75].

In conclusion, exposure to CILD risk factors during development and early life may not only alter the structure and function of the lung, but may also pre-determine the response to certain therapeutic drugs (Figure 3). However, besides cigarette smoke or house dust mite allergens the effect of other CILD risk factors on epi-genetic events has not been investigated, due to the lack of funding. The most frequent argument by research funding bodies to reject such studies is the lack of evidence.

Cigarette Smoking and CILD

The increase of COPD that has been reported worldwide over the past decades is of course due to cigarette smoking but other risk factors such as fine dust, organic dust or fine ashes are now being recognized as important co-factors which are essential to trigger the pathology [76,77]. Cigarette smoking of mothers during pregnancy has been identified as a higher risk for their children to develop CILD later in life than the exposure to cigarette smoke after birth. Stick et al examined 500 healthy children in Western Australia and found that cigarette smoking during pregnancy was the strongest risk factor for reduced lung function after birth [78]. The outstanding impact of smoking during pregnancy and the development of asthma or other chronic respiratory symptoms later in life has been supported by a study in Russia in a cohort of 5,951 children aged 8-12 years with the strongest effect of mothers smoking during pregnancy on asthma, chronic bronchitis and wheezing [79]. In Brazil, a study including 1,302 children confirmed that smoking during pregnancy was a more potent risk factor for asthma at the age of 7 years than second hand smoke and furthermore, the exposure to inhaled particles <pm10 had no effect on respiratory diseases [80]. A nationwide study in Japan including 40,580 children aged 6 months to 8 years reported a 45% increase of asthma-like symptoms in families with both parents smoking during pregnancy and after birth [81]. Several studies compared passive to active smoking of mothers during pregnancy and after birth confirming that smoking during pregnancy have more severe lasting effect for their children developing respiratory symptoms and asthma later in life [82-84]. These studies were supported by a meta-analysis including 79 prospective studies of passive smoking during pregnancy and after birth [85].

Unsurprisingly, many investigators suggested that the reduction of exposure to risk factors may prevent the disease initiation. This view however may be too simplistic as there is evidence that the predisposition to develop COPD is handed down through the maternal line over at least two generations [7,8,86]. In humans, it had been reported that smoking of grandmothers or mothers has equal impact to increase the susceptibility of grandchildren/children to develop CILD [7,86,87]. The later study by Magnus et al included a total of 3,013 grandmothers and 23.5% of them had reported smoking during their pregnancy with the mothers of the second generation children. This hypothesis has been strengthened by the observation that in a rat model, the pre-condition to develop asthma-liked symptoms has been induced by nicotine exposure of the grandmother generation [28]. The mechanisms underlying this kind of inheritance are not well understood and only a few experimental studies were able to hint how this epigenetic gene regulation is handed down through at least two generations [88]. In conclusion, the biggest risk factor to develop CILD which include asthma, COPD and wheezing is smoking of mothers during pregnancy, while exposure to cigarette smoke, dust or ashes of the mothers during pregnancy has a much lower impact for the child’s lung function later in life. The existing data however does not allow drawing conclusion if there is a specific time window during pregnancy when cigarette smoking of mothers is specifically harmful to the development of the embryo’s lung.

Stress and CILD

Asthma attacks can also be induced by physical or psychological stress for which the mechanism is completely unknown. Stress of the mothers during pregnancy has a directive effect on embryo development and on stem cell imprinting, suggesting that epigenetic mechanisms are determined in specific time windows [89]. Three recent studies indicate that distress of mothers during pregnancy has an impact on asthma development later in life for the children. One study from Canada investigating children of 12 years even claimed that stress of mothers during pregnancy had a higher effect in girls than boys regarding respiratory symptoms [90]. Atopic disorders including asthma, wheeze, allergic rhinitis, circulating IgE and dermatitis were increased in the children of mothers exposed to stress during pregnancy [91], and a similar result was reported by Van de Loo [92]. Long lasting stress situations during pregnancy seem to affect lung development and maturation during embryogenesis but the data is to scarce to draw any conclusion on the mechanism. The most probable scenario is that stress hormones produced by the mother affect the lung development through hormone receptors which are essential to determine cell differentiation as it has been reported in animal models and human clinical studies [53,93,94].

Molecular Biology Linked to CILD Susceptibility?

DNA Methylation can be induced by cigarette smoking across the human genome which lasts even years after smoking cessation [95]. Three recent publications reported that prenatal smoking of mothers induces DNA Methylation which seems to be irreversible during the life time of the child [14,15,96]. Comparing the effect of cigarette smoking of mothers during pregnancy in 65 children with asthma to that of 462 children also with asthma born to mothers who did not smoke during pregnancy, significant CpG methylation was observed in 2 genes (FRMD4A, Clilorf52) and a lower increase of methylation in 4 additional genes (XPNPEP1, PPEF2, SMPD3, CRYGN) [14]. The function of these genes is either unknown of affects protein phosphorylation; not much is known on the function of these...
proteins by cigarette smoke beside this study. The study suffers from the lack of healthy control groups, which would consist of healthy children from mothers who smoked and did not smoke during pregnancy. A second study supported the effect of tobacco smoke during pregnancy on DNA methylation in 572 children. Smoking of mothers during pregnancy reveal increase DNA methylation of the children at school age and the analysis of 26 CpG loci indicated a gene specific methylation pattern including the AHRR and CYP, which are both related to inflammation innate immunity and DNA accessibility [15]. Most of the other loci which were methylated by prenatal smoking have no known function, therefore, their impact on lung function and development has to be further investigated.

DNA Methylation and Histone modification are two of the best studied epigenetic regulatory mechanisms that are associated with organ function efficiencies [97,98]. Interestingly, DNA methylation induced by cigarette smoking affected α-1 anti-trypsin deficiency, which may be linked to the development of emphysema [99]. In this study, CpG methylation occurred mainly in genes that regulate signal transduction proteins inducible by TGF-β. Cigarette smoke also inhibits intra-cellular signaling of Erk1/2 in human lung fibroblasts [100], which also regulates mucus secretion in a rat model [101]. Protein Arginine Methyl Transferase 1 (PRMT1) modifies Histones 3 and 4 and can act as a transcription factor. Suggested by an animal model PRMT1 expression shifts from epithelial to mesenchymal cells as the result of chronic exposure to asthma stimuli [102] and modifies remodeling properties of both airway wall fibroblasts and smooth muscle cells [103]. In asthma, constitutive high expression of caused by decreased expression of microRNA19a and subsequent up-regulation of Erk1/2 MAPK had been linked to increased remodeling of isolated bronchial wall mesenchymal cells [104].

Another mechanism that regulates DNA accessibility is the acetylation of histones, which has been suggested to play an important role in CILD [105,106]. Pro-inflammatory signaling such as NFkB and its inhibitors IκB were linked to histone acetylation in animal models [107,108]. Other modification of histones include phosphorylation and sumoylation [109,110]. However, the question why DNA methylation or histone acetylation induced by cigarette smoke seems to be restricted to certain genes is not understood.

A recently reported novel post translational modification factors are Protein Arginine Methyltransferases (PRMT) [111]. The function of PRMT had been linked to histone methylation and thus could affect DNA accessibility indirectly [112,113]. In an animal model, the cell type specific expression of PRMT1 was described [102]. In this model, PRMT1 was induced in the bronchial and alveolar epithelium after allergen inhalation. Furthermore, it was shown that IL-4 was the major mediator of the allergen effect on PRMT1 expression [102]. In the second study of the same group, it was reported that PRMT1 expression is cell specific with being up-regulated in acute inflammation after allergen exposure in the epithelium, while in animals with chronic allergen exposure, it is up-regulated in fibroblasts [114]. In fibroblasts, PRMT1 correlated with the expression of COX-2 and VEGF and function as a mediator of TGF-β stimulation. Finally we provided evidence that PRMT1 is constitutively up-regulated by an epigenetic event which diminishes the expression of the Erk1/2 MAPK inhibitor microRNA-19a in human airway smooth muscle cells of asthma patients and increases airway smooth muscle cell proliferation, migration and inflammation [114]. Furthermore, we show that PRMT1 affects the activity of mitochondria, thus cell activity and energy consumption through up-regulated PGC-1a expression, which is an epigenetic mechanism by itself.

**Conclusion**

Despite the huge number of epidemiological studies providing evidence for the correlation of smoking during pregnancy and preterm birth as the two major risk factors to develop CILD later in life, the underlying mechanism(s) is not well understood. New studies suggest that the susceptibility to develop asthma and COPD may not be inherited through a genetic pre-condition, but rather be inherited through irreversible epigenetic events. Future studies have to investigate the mechanisms that “fix” epi-genetic modifications and make them “inheritable” (Figure 4).

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make epi-genetic events "inheritable". Preventive strategies have to be developed and pregnant women have to be informed about the risk factors which pre-dispose their children to suffer from lifelong malfunction of the lung. Strategies to reduce the burden of CILD can only be achieved by projects that link basic, clinical and epidemiological research. However, as long as the public is not aware of the problem, such studies will have difficulties to obtain funding.

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