Genetic and Environmental Determinants of Respiratory Health
Zeng, Xiang

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2016

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 12-01-2020
CHAPTER 8

Summary, discussion and future perspectives
Summary

The aim of this PhD project was to assess whether environmental exposures, such as electronic waste (e-waste) exposure, occupational exposure and air pollution exposure, are associated with the level of lung function and the prevalence of respiratory symptoms. In addition, we also were interested in evaluating differences in genetic susceptibility to these exposures between individuals. The studies described in this thesis have shown that environmental exposures are associated with lower lung function levels and a higher prevalence of respiratory symptoms. We identified several genetic polymorphisms that are associated with individual susceptibility to occupational and nitrogen dioxide exposure.

Chapter 1 introduces the study. The environmental risk factors of interest are explained and more information about the used outcome measures is given. In addition, the methods of studying genetic associations are described. Finally, the study populations used in the thesis are introduced.

Chapter 2 reviews studies investigating the adverse effects of e-waste on the human body. We assessed cross-sectional associations between e-waste exposures to toxic heavy metals and organic pollutants and impaired human health including endocrine toxicity, respiratory toxicity, developmental toxicity, reproductive toxicity, neurotoxicity, and genetic toxicity. In this study, we concluded that heavy metals and organic pollutants derived from e-waste can accumulate in environmental media such as air, soil, dust, water, and in the human body directly by inhalation, ingestion and dermal absorption, or indirectly by the food chain. Exposure to e-waste has adverse effects on human health such as endocrine disturbances, decline in lung function, developmental delay, sperm damage, mental decline, and DNA and protein damage.
Chapter 3 describes a review in which we assessed the adverse effects of a number of heavy metal pollutants from an e-waste recycling area on the health of children, including respiratory, cardiovascular, nervous, immune, reproductive, skeletal, and urinary health. Children can become exposed to heavy metals in air, dust, soil, sediment, water, and food sources through several routes that include ingestion, inhalation, and dermal absorption from combustion, discharges and manufacturing facilities. Children are considered more susceptible to hazardous metal substances compared to adults for several reasons such as excess routes of exposure, a higher basal metabolic rate, and a lower toxin elimination rate. We found that the concentration of heavy metals in the environmental media such as air, dust, soil, sediment, and water is higher in an e-waste exposed area than in the reference areas. Living in e-waste exposed areas is associated with higher heavy metal concentrations in children’s blood and heavy metal exposure is associated with body system impairment.

Chapter 4 describes a study in which we assessed the association between living in an e-waste exposed area and levels of heavy metals in the blood in pre-school children. In addition, we investigated the association between blood heavy metals levels and the level of lung function, and the association between living in an e-waste exposed area and level of lung function in these children. We conclude that living in e-waste exposed areas is associated with higher levels of blood lead, platelets and thrombocytocrit, and lower levels of hemoglobin, hematocrit and lung function. In addition, hemoglobin was associated with higher lung function levels.

Chapter 5 describes a number of genome-wide association studies in which we aimed to identify and replicate novel genetic loci that associated with respiratory symptoms including cough, dyspnea and phlegm. We performed an identification analysis in 7,976 subjects from the first data release of the LifeLines cohort study (LifeLines I), and replicated our initial
findings in 5,260 subjects from the second data release of the LifeLines cohort study (LifeLines II) and in 1,529 subjects from the Vlagtwedde-Vlaardingen cohort. We identified and replicated one SNP rs16918212, located on A2MP1 associated with cough. However, the gene A2MP1, a pseudogene, has not been associated with lung function impairment or respiratory diseases in literature. Therefore, this gene might not be a plausible gene underlying the development of respiratory symptoms.

Chapter 6 describes a genome-wide interaction study in which we aimed to identify novel susceptibility loci that affect individual susceptibility to common occupational exposures, i.e. vapors, gas, dust or fumes (VGDF), biological dust, mineral dust, gas and fumes, pesticides, herbicides, insecticides, aromatic solvents, chlorinated solvents, other solvents, and metals, on respiratory symptoms including cough, dyspnea and phlegm. First, we performed an identification analysis in 7,976 subjects from the first data release of the LifeLines cohort study (LifeLines I), then verified our initial findings in 5,260 subjects from the second data release of the LifeLines cohort study (LifeLines II) and in 1,529 subjects from the Vlagtwedde-Vlaardingen cohort using meta-analysis of the interaction effects. Additionally, we assessed whether these SNPs were cis-eQTLs in lung tissue. We identified and replicated 11 SNPs that interacted with one of the occupational exposures. Several identified SNPs were located in genes that may be associated with respiratory health, i.e. TMPRSS9, TOX3 and ARHGAP18. Of these 11 replicated SNPs, 3 SNPs were cis-acting eQTLs associated with gene expression of their neighboring genes FCER1A, CHN1 and TIMM13. Further research should assess whether the identified genes are true genetic loci for the development of respiratory symptoms.

Chapter 7 describes a genome-wide interaction study in which we aimed to identify novel susceptibility loci that affect individual susceptibility to NO2 exposure on respiratory symptoms including cough, dyspnea,
phlegm and wheeze. SNP by NO$_2$ exposure interactions were assessed in 10,762 subjects from the LifeLines cohort study. Furthermore, we assessed whether these SNPs that interacted with NO$_2$ on respiratory symptoms were cis-eQTLs in lung tissue. Several identified loci were plausible genes associated with lung function levels in the literature, i.e. ROCK2, GLS and KCTD10. Of the 32 identified SNPs, 15 SNPs were cis-eQTLs associated with gene expression of their nearby genes such as DBF4B, PRKAG2, EPS15, GRPEL1, MMAB, GLS, ZNF25, NCKAP5, GABRA2, ROCK2, MON1B and C14orf4 in lung tissue. The most interesting SNPs was located in the gene ROCK2. We found that subjects carrying the minor allele of the SNP had a significantly higher ROCK2 gene expression in lung tissue and also had a greater risk to wheeze in association with NO$_2$ exposure, compared to subjects carrying the 2 major alleles of the SNP. This data provides additional support to explore a possible role of these genes in the development of respiratory symptoms. Further studies should replicate these results in other independent cohorts, and determine whether the identified genes are true susceptibility loci for respiratory symptoms due to exposure to ambient NO$_2$, and whether these SNP-by-NO$_2$ exposure interactions consequently increase the risk to develop lung diseases such as asthma and COPD.

**Discussion**

**Environmental exposure and respiratory symptoms and levels of lung function**

*E-waste exposures and respiratory symptoms and levels of lung function*

Electronic waste (e-waste), from electrical and electronic equipment or products after reaching the end of their useful life, has become the largest growing amount of waste in the world (1, 2). It was estimated that global e-waste generation was 41.8 million tons in 2014 and may increase to 65.4 million tons by 2017 (3). E-waste contains heavy metals and plastics that can be released to the environment during inappropriate recycling
processes and disposal, which could be harmful for humans, animals, vegetation or other environmental materials (4-6). Our recent study showed that levels of lead and cadmium both in PM$_{2.5}$ and blood were higher from the e-waste exposed area than the reference area, and prevalence of respiratory symptoms including cough, dyspnea, phlegm and wheeze was higher in the e-waste exposed area than the reference area (7). Living in e-waste exposed areas was associated with higher heavy metal concentrations in blood including lead and cadmium, and a higher prevalence of respiratory symptoms including cough, dyspnea, phlegm and wheeze in preschool children (7). One of our previous studies showed that the blood levels of three transition metals, i.e. chromium, manganese, and nickel, were higher in children living in the e-waste exposed area than the reference area, but there was no difference in forced vital capacity (FVC). However, in the youngest age group (8-9 years) FVC was significantly lower in children from the e-waste exposed area compared to the reference area (8). This indicates that young children may be sensitive and vulnerable to e-waste exposure because of additional routes of exposure, high-risk behaviors, and their changing physiology compared to older children and adults (1). Therefore, in chapter 4 we performed another study measuring concentrations of lead and cadmium in blood, and lung function levels (FVC and forced expiratory volume in 1 second (FEV$_1$)) in very young (i.e. preschool) children from the e-waste exposed area and two reference areas. We found that preschool children from the e-waste exposed area have higher concentrations of lead in their blood and lower lung function levels when compared to the reference areas. Furthermore, we assess whether there were associations between concentrations of lead and cadmium in blood, and lung function levels in preschool children, and we found that there was no significant association between them. However, living in the e-waste exposed area was associated with higher lead levels in blood and lower lung function levels in preschool children, which may confirm our previous hypothesis that young children are more susceptible to develop lung function impairment when exposed to e-waste compared to older children.
Occupational exposures and respiratory symptoms and lung function levels

In the studies presented in this thesis, occupational exposures have been divided into four categories: Vapors, gas, dust or fumes (VGDF), including the subcategories biological dust, mineral dust, and gases and fumes; Pesticides, including the subcategories herbicides and insecticides; Solvents, including the subcategories aromatic solvents, chlorinated solvents and other solvents; and Heavy metals such as lead, cadmium, chromium, mercury and nickel (Table 1). Previous studies have shown that VGDF exposure is clearly associated with lower levels of FEV$_1$ and FEV$_1$/FVC, and a higher prevalence of large airway obstruction in a sample from the general population (9-11). Small airways are affected by occupational exposure to VGDF, and the subcategories biological dust, gases and fumes as well (12). It is worth to mention that these associations also exist in subjects with normal FEV$_1$/FVC and FEV$_1$% predicted values, demonstrating that the effects of VGDF exposure on the small airways are independent of their effects on the large airways (13). Occupational dust exposure is associated with respiratory symptoms including chronic cough, phlegm, and wheeze in general population studies conducted in Europe (13-15), China (16, 17), and USA (18, 19). Specifically exposure to biological dust is associated with a higher risk of emphysema and COPD in the general population (20). Mineral dust exposure is associated with a lower level of FEV$_1$ and a higher COPD prevalence. Occupational exposure to gases and fumes is associated with airflow obstruction, respiratory symptoms including chronic cough, phlegm and persistent wheeze, and a lower FEV$_1$/FVC and a lower FEV$_1$% predicted values compared with subjects without occupational exposure in both men and women (21).
Table 1. Most prevalent occupations in subjects exposed in the LifeLines sample (table modified from De Jong (9,22,44)).

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Jobs</th>
</tr>
</thead>
<tbody>
<tr>
<td>VGDF</td>
<td>Dairy and livestock producers, Carpenters, Freight handlers, Bakers and pastry-cooks, Crop and animal producers (mixed), Veterinary assistants, floor layers and tile setters, motorize farm and forestry plant operators</td>
</tr>
<tr>
<td>Biological dust</td>
<td>Welders and flame-cutters, Agricultural and industrial mechanics and fitters, Freight handlers, Gardeners, horticultural and nursery growers, Building and construction laborers, Earth moving and related plant operators, Field crop and vegetable growers, Crop and animal producers (mixed), Construction and maintenance laborers (roads), and Earth moving and related plant operators</td>
</tr>
<tr>
<td>Mineral dust</td>
<td>Heavy truck and lorry drivers, Motor vehicle mechanics, Welders and flame-cutters, Agricultural and industrial mechanics and fitters, Plumbers and pipe fitters, Painters, Butchers, fishmongers and related preparers, and Earth moving and related plant operators</td>
</tr>
<tr>
<td>Gases and fumes</td>
<td>Field crop and vegetable growers, Gardeners, Horticultural and nursery growers, and Tree and shrub crop growers</td>
</tr>
<tr>
<td>Pesticides</td>
<td>Field crop and vegetable growers, Gardeners, Horticultural and nursery growers, Tree and shrub crop growers, and Mixed crop and animal farmers</td>
</tr>
<tr>
<td>Herbicides</td>
<td>Painters, Printing machine operators, Varneshers and related painters, Motor vehicle mechanics, Agricultural and industrial mechanics sheet metal workers</td>
</tr>
<tr>
<td>Insecticides</td>
<td>Welders and flame-cutters, Motor vehicle mechanics, Agricultural and industrial mechanics, Sheet metal workers</td>
</tr>
<tr>
<td>Solvents</td>
<td></td>
</tr>
<tr>
<td>Aromatic solvents</td>
<td></td>
</tr>
<tr>
<td>Chlorinated solvents</td>
<td></td>
</tr>
<tr>
<td>Heavy metals</td>
<td></td>
</tr>
</tbody>
</table>

Our previous study showed that occupational pesticide exposure was associated with a lower level of lung function (FEV$_1$ and FEV$_1$/FVC) and accelerated decline of lung function (FEV$_1$ and FEV$_1$/FVC) consistent with airway obstruction as measured by spirometry in two general population cohorts (22). These associations with pesticides were seen in both men and women and smokers and non-smokers. A series of studies conducted within the Agricultural Health Study, North Carolina showed a
higher prevalence of wheeze, chronic bronchitis and asthma both in the pesticide applicators (farmers) (23-26) as well as in their spouses (27-29). A few small scale cross-sectional studies found associations between specific types of pesticides and lower levels of FEV₁ and FVC in occupationally exposed farmers from Sri-Lanka (30), South Korea (31), Costa Rica (32) and Spain (33). Globally, over 5 billion pounds of pesticides were used in 2006 (34), and more than 1.1 billion workers (approximately 34% of the global working force) were exposed to pesticides in the agricultural sector (35). People living in agriculture-intensive regions may be at risk of pesticide drift exposure from neighboring farms (36). The Netherlands is famous for its high-density population and high-yield agricultural production areas where large numbers of people live near greenhouses and pesticide-treated farmland, and may be at risk of pesticide-related conditions or illnesses such as a lower lung function and a higher prevalence of respiratory symptoms or diseases. To date, knowledge on the association between occupational pesticide exposure and respiratory symptoms is still limited and not fully understood.

Most occupational solvents are complex substances containing a large number of individual specific molecules. For instance, the solvents referred to as “aromatic” are composed primarily of aromatic constituents containing one or more benzene rings. Respiratory and other health effects of solvent exposure depend on the actual inflicted exposure dose. Higher levels of solvent exposure tend to lead to a higher risk of suffering from respiratory symptoms (37). According to the Agricultural Health Study, occupational exposure to solvents from painting and cleaning were associated with higher odds of wheeze in a duration-dependent fashion (38). A cross-sectional study, conducted in gun factory workers, investigated the association between exposure to solvents and asthma-related symptoms including chronic cough, chronic bronchitis and dyspnea. Asthma-related symptoms were more prevalent in exposed workers than unexposed, both in non-smokers and in smokers (39). A systematic review investigating the effects of occupational exposure to formaldehyde on respiratory symptoms and lung function, which showed
that occupational exposure to formaldehyde was associated with a higher prevalence of cough, dyspnea and wheezing in most of the included studies (40). Postnatal exposure to solvent-containing products in the home was associated with a higher risk of wheezing, which remained after adjustment for prenatal exposure (41). Exposure to aromatic solvents, chlorinated solvents and any solvents were associated with the risk of small for gestational age (fetal growth restriction) in a population-based sample of women in the National Birth Defects Prevention Study (42). Preterm babies are likely to have a lower birth weight compared to babies with normal birth conditions, and a low birth weight in children born at term was associated with a higher risk of respiratory symptoms (43). Interesting, occupational exposure to chlorinated solvents was significantly contributing to chronic mucus hypersecretion (CMH) in subjects without COPD, but not at all in subjects with COPD (44).

Occupational exposure to metals is not limited to mining and smelting industries. Metals are also needed for the industrial development and occupational exposure to metals can occur in a diverse spectrum of jobs involved in manufacturing, processing, and application of metals and metal-containing products (45). Previous cohort studies from Norway found that exposure to heavy metals was associated with lower lung function levels, increased lung function loss, and higher prevalence of respiratory symptoms in the same worker population (46-48). Exposure to metalworking fluids was associated with a higher risk of cough, dyspnea, wheezing, chronic bronchitis, and asthma in occupationally exposed workers from southern Finland (49). Environmental heavy metals not only cannot be degraded in the environment, but also can be enrich in humans through ingestion, inhalation, dermal absorption, and food chain. Previous cross-sectional studies conducted in China demonstrated that heavy metal levels such as lead and cadmium in environmental media including air (7, 50), soil (51), dust (52) and water (53) and in blood of children (7) were higher for the e-waste recycling area compared to the reference area. We found that blood chromium was associated with a higher prevalence of cough, and blood manganese was associated with a
higher prevalence of wheeze (7). In addition, other cross-sectional studies showed blood lead and cadmium levels were associated with a lower lung function in adults (54, 55). Finally, in a large general population-based study, CMH was associated with occupational heavy metal exposure in subjects without COPD, but not in subjects with COPD (44).

Nitrogen dioxide (NO\textsubscript{2}) is one of the major gaseous air pollutants that continue to raise concern. Traffic pollution is the main outdoor source of NO\textsubscript{2} (56). The most important indoor sources of NO\textsubscript{2} include tobacco smoke and gas-, wood-, oil-, kerosene- and coal-burning appliances such as stoves, ovens, gas and water heaters and fireplaces, and particularly poorly maintained appliances. Outdoor NO\textsubscript{2} from natural and anthropogenic sources and air exchange rates between outdoor and indoor can also influence indoor levels (57). Ambient concentrations of NO\textsubscript{2} vary widely according to local sources. Several studies reported that ambient NO\textsubscript{2} exposure has been associated with lower lung function (58-60), respiratory symptoms (61-63), hospital admissions and mortality (64-66). In a German birth cohort, a higher annual average NO\textsubscript{2} concentration was associated with more bronchial symptoms of children in the first year of life (67). A review showed that long-term NO\textsubscript{2} exposure (below 40 µg NO\textsubscript{2}/m\textsuperscript{3} World Health Organization limit) was associated with adverse effects including respiratory symptoms/diseases (59). Outdoor NO\textsubscript{2} exposure was Vgests that girls may be more susceptible to indoor NO\textsubscript{2} than boys (62). However, the associations were not consistent in all studies. For instance, the large PEACE study found no consistent effects of outdoor NO\textsubscript{2} on respiratory symptoms or lung function (68). There has been increasing interest both in outdoor NO\textsubscript{2} pollution and in indoor NO\textsubscript{2} pollution in the recent decades, and studies showed that the outdoor NO\textsubscript{2} exposure was higher than indoor NO\textsubscript{2} exposure in the same area (69-72). One of our previous studies showed that higher long-term NO\textsubscript{2} concentrations were associated with lower post bronchodilator FEV\textsubscript{1} and FVC % predicted (73).

**Genetic risk factors and respiratory symptoms and levels of lung**
function

GWA Studies

A GWA study is a hypothesis-free approach to identify genetic variations associated with a trait or a disease of interest (74). Compared to candidate gene studies focusing on specific known genes, the advantage of GWA studies is that there is no pre-selection of genes and thus novel susceptibility loci associated with a disease or trait can be identified across the entire genome. Although GWA studies were successful in identifying new loci associated with lung function and lung diseases such as asthma and COPD, the newly identified genetic variants have so far only explained a small proportion of the genetic contribution to these complex diseases or traits, and largely disregard environmental factors that may trigger the development of these diseases and traits (75). As we know, respiratory symptoms are associated with accelerated lung function decline (76-78), lung diseases (79-81) and mortality (82-84). To date, only one GWA study on respiratory symptoms (i.e. CMH) has been published (85). Our GWA study (chapter 5) did not identify SNPs that were genome wide significantly \((p < 2.06 \times 10^{-7})\) associated with the risk of cough, dyspnea and phlegm but we did identify 17, 19 and 14 SNPs associated with cough, dyspnea and phlegm respectively, at a \(p\)-value < \(10^{-4}\). However, these SNPs did not show a convincing association with the presence of respiratory symptoms in the meta-analysis of two independent replication cohorts. This lack of replication can be explained by the fact that respiratory symptoms can be caused by different environmental exposures or can be a presentation of different underlying diseases with specific genetic or environmental origins. Susceptibility to environmental exposures may be genetically determined and susceptibility loci may differ between exposures. The environment should thus be taken into account when studying the association between genetics and respiratory symptoms. Therefore, the next logical step is to perform a genome-wide interaction (GWI) study to identify genetic loci for respiratory symptoms in the interaction with known harmful environmental exposures.

GWI Studies
To date, few studies have comprehensively examined combined genetic and environmental effects on lung function (75, 86, 87), and no study has comprehensively examined the combined genetic and environmental effects on respiratory symptoms. A GWI study is a hypothesis free approach that is used to identify genetic loci that affect the susceptibility for the effects of known harmful exposures on a trait or a disease. Compared to a GWA study identifying genetic variations predisposing for a trait or a disease, a GWIs study is aiming to identify genetic loci based on gene-environmental exposure interactions on a trait or a disease across the entire genome (86). Hancock et al (2012) reported one of the first genome-wide gene-environment interaction studies. In this study the environmental interacting factor (smoking) and the study identified novel loci associated with the level of lung function (FEV$_1$, FEV$_1$/FVC) that would have been missed when only focusing on direct genetic effects (87). It is likely that genetic susceptibility is also of importance for occupational exposures and air pollution such as ambient NO$_2$ exposure. So far, there was no GWI study identifying genetic loci that affect the susceptibility for the effects of environmental exposures such as occupational exposure and ambient NO$_2$ exposures on respiratory symptoms.

Therefore, in this PhD project we aimed to assess the inter-individual difference in susceptibility for respiratory symptoms including cough, dyspnea, phlegm and wheeze when exposed to occupational exposures and ambient NO$_2$. With the studies presented in this thesis we are the first assessing gene-environment interaction effects on respiratory symptoms in a genome-wide hypothesis-free manner. The results show that the identified genetic variants based on gene-environmental exposure interactions are associated with respiratory symptoms; therefore they are plausible candidate genes. In order to obtain additional insight in the biological plausibility of these genes and potential pathways engaged in the development of respiratory symptoms, we have performed gene expression analysis. In chapter 6 we have performed a gene expression analysis and found that 4 of the SNPs in interaction with biological dust and mineral dust exposure were cis-eQTLs in lung tissue. In chapter 7 we
Genetic and environmental determinants of respiratory health

performed a GWI analysis of gene by ambient NO₂ exposure interaction, and found that 14 of the SNPs in interaction with ambient NO₂ exposure exposures were cis-eQTLs in lung tissue. These findings suggest that these SNPs may affect individual susceptibility to occupational exposures and ambient NO₂ exposure by altering gene expression levels. The other SNPs identified in the GWI study were not cis-eQTLs in lung tissue, but may affect individual susceptibility to occupational exposure and ambient NO₂ exposure via other mechanisms, i.e. changed protein structure, altered miRNA levels or methylation. Determining the functional mechanisms of the identified genetic variants will be an important focus in future studies.

Future perspectives

Epidemiological studies

In this thesis we have shown that environmental exposures including e-waste exposures, occupational exposures and ambient NO₂ exposures are associated with the level of lung function or the prevalence of respiratory symptoms. In addition, the GWI studies presented in this thesis have produced several novel loci which are associated with the development of respiratory symptoms. However, several important issues remain to be investigated. First, association between environmental exposures and the level of lung function and the prevalence of respiratory symptoms were studied in cross-sectional settings. In chapter 4 we investigated associations between e-waste exposures and the lower level of lung function in the cross-sectional study, which prevents us from drawing a causal relationship conclusion for the nature of cross-sectional study. Longitudinal studies are needed to assess associations between environmental exposures and the level of lung function and the development of respiratory symptoms in the future. Second, the genome-wide interaction studies presented in this thesis have focused on the prevalence of respiratory symptoms. Individuals with respiratory
symptoms are more apt to have lower level of lung function. In order to fully illustrate the pathways leading to the development of respiratory symptoms, future studies should focus on the level of lung function as well. Finally to answer the question why and how non-exposed individuals or low exposure individuals develop respiratory symptoms, associations between genes and their interactions on lung function levels and respiratory symptoms prevalence should be studied in a subgroup including only non-exposed individuals or low exposure individuals. GWA or GWI studies in these settings will teach us whether the same or different pathways underlie exposed and non-exposed respiratory symptoms such as occupational and non-occupational respiratory symptoms.

Post-GWAS functional studies

Genome-wide approaches provided the opportunity to identify genetic loci at which common variants influence symptom and disease risk or quantitative traits cross the entire genome. As an example, GWA and GWI studies could provide information on genes involved in previously unsuspected pathways, which could have been never considered in a candidate gene approach. Although GWA and GWI studies can provide a comprehensive list of significantly associated genetic variants, they are not designed to pinpoint the causative genes or gene mutations that underlie the complex trait or disease of interest, and only can explain a small fraction of the heritable component of symptoms/disease risk and the phenotypic variation that is observed. If they are additionally located outside the coding region of the gene, they also provide little evidence in terms of their functional significance (88). Therefore, several approaches have been used to explore and establish the functionality of identified SNPs and whether genes are causative (88, 89): (1) eQTL analysis is used to explore the association between gene variants and mRNA expression levels; (2) functional validation approach to establish the functional significance with in vitro and/or in vivo overexpression or
knock-down biological experiments; (3) bioinformatics combining epidemiologic, genomic, epigenetic, metablolomic and proteomic information for network analysis will help exploit integrative genomic approaches according to available databases. In spite of such attempts, identification of causative genes/mutations has been quite difficult and remains very challenging.

With the studies presented in this thesis we are the first assessing genome-wide interactions with environmental exposures such as occupational exposures and ambient NO₂ exposure in relation to the presence of respiratory symptoms. We identified several novel loci. Future challenges will be to understand the function of these loci. There are several options to go from identifying SNPs to assessing their functional mechanisms. The identified genes from our GWI studies still need investigation regarding their functionality in humans. In chapter 6 and chapter 7 of this thesis we have assessed whether identified SNPs were cis-eQTL in lung tissue, which gave us additional insight in potential pathways underlying the observed associations. Other approaches for post-GWA study analyses include re-sequencing, experimental models and epigenetic mechanisms (90, 91). Re-sequencing or fine mapping can be conducted sequence-based in follow-up of a GWA study, and can be used to identify the causal SNP that is in the linkage disequilibrium with the SNP associated with the outcome under study. In addition, fine mapping in different populations may also be used to identify rare variants associated with respiratory symptoms, thereby potentially explaining some of the missing heritability of the respiratory symptoms. Overexpression or knock-down of a gene in animals or cells may contribute to better understanding of biological pathways leading to a trait or a disease of interest. Epigenetic mechanisms including DNA methylation, histone modification and non-coding RNA (ncRNA) can contribute to gene regulation, and may mediate associations between SNPs and disease found in GWA or GWI studies.

Epigenetics
Epigenetics refers to heritable changes in gene expression that do not involve changes to the underlying DNA sequence, resulting in a change in phenotype without a change in genotype (92). Epigenetic mechanisms occur regularly and naturally, but can also be influenced by several factors like age, environment, and lifestyle and disease state (93). Epigenetic alterations mediate genomic adaption to the environment and can contribute to the development of disease phenotypes, as can genetic variants. GWA studies or GWI studies are not able to provide evidence for causative genetic effects of respiratory symptoms and diseases and can not fully explain the heritability of human. Genome-wide epigenetic studies can provide new insights into respiratory symptoms and diseases. Epigenetic mechanisms including DNA methylation, histone modification, nucleosome remodeling and non-coding RNA (Figure 1), are essential for normal development and differentiation, but also may be an important link between exposures and the development of respiratory symptoms or airway diseases (94-96). So far, most epigenetic studies focused on the effects of smoking on diseases, and genome-wide methylation patterns were shown to be associated with smoking status (97-99). In addition, cigarette smoke has been associated with both histone modifications (100) and altered expression of miRNA (101). Studying the role of epigenetic mechanisms in mediating associations between environmental exposures and gene expression and in the development of respiratory symptoms will be important in further studies. Such studies may improve our understanding of biological pathways underlying respiratory symptoms.
Figure 1. Major epigenetic mechanisms controlling gene expression (picture modified from Hagood (102)).

In summary, combining and analyzing genotype, methylation, gene expression and phenotypic data will be useful to uncover mechanisms underlying respiratory outcomes and diseases in future studies. This will require large computational power for genome-wide data. Soon, whole-genome methylation data of a subsample of 1,600 subjects from the LifeLines cohort study will be available, which will allow us to assess DNA methylation, and gene-environment interactions in relation to the development of respiratory symptoms or diseases. This may contribute to early identification of groups at risk for developing respiratory symptoms or disease, and might allow intervention at a very early stage of disease. The advances in GWI studies and in genome-wide epigenetic studies will probably contribute to the discovery of novel biological mechanisms underlying disease development and a better understanding of the etiology of respiratory diseases.
References


98. Joubert BR, Felix JF, Yousefi P, Bakulski KM, Just AC, Breton C,


