Chapter 1

General Introduction
Asthma and COPD are highly prevalent, obstructive airways diseases. Asthma is a chronic inflammatory airways disease, affecting approximately 300 million people worldwide(1). The global prevalence of asthma is estimated to be around 4.3% but varies widely across countries, highest prevalences being reported in developed countries (e.g. 15.3% in The Netherlands) and lowest prevalences in developing countries (e.g. 2.9% in Bangladesh)(1). The disease is characterized by recurrent attacks of dyspnea, cough and wheeze. Asthma leads to decreased quality of life and contributes significantly to overall healthcare costs(2,3). COPD is the third leading cause of death worldwide according to the World Health Organization(4). Important characteristics of the disease are irreversible and progressive airflow obstruction due to airway inflammation, airway narrowing and loss of lung tissue. Cigarette smoking is the major cause of COPD, but other noxious particles such as air pollution and biomass fuel used for cooking are currently acknowledged as important risk factors(5). Both asthma and COPD are considered heterogeneous diseases in terms of clinical presentation, underlying inflammatory profiles and environmental factors influencing disease severity. Disentangling the heterogeneity of obstructive airways diseases is an important field of research, which eventually can lead to development of tailored treatment for each individual patient. In this thesis, we aimed to investigate the heterogeneity of asthma and COPD by focusing on clinical and molecular phenotyping.

**CLINICAL PHENOTYPING**

**Airway hyperresponsiveness; a hallmark of asthma?**

*Prevalence of AHR*

Airway hyperresponsiveness (AHR) is a key feature of asthma. It is defined as exaggerated airway narrowing upon exposure to non-specific stimuli, such as cigarette smoke, cold air or perfumes. Although AHR is usually accompanied by respiratory symptoms, it can also be present in asymptomatic subjects, so-called asymptomatic AHR. It is estimated that the prevalence of asymptomatic AHR varies between 2.2 to 14.3% in the general population(6). In the past decades, several explanations for asymptomatic AHR have been put forward such as increased airway inflammation, airway remodeling and altered perception of symptoms(7–9). Of importance, the presence of asymptomatic AHR is not an innocent feature, but is associated with the development of asthma later in life(10,11).

*Measurements of AHR*

The first report describing the use of inhaled acetylcholine to measure AHR was published by Robert Tiffeneau in 1945(12). Since then, techniques to measure AHR have been
improved. Nowadays, measurement of AHR is performed using a provocation test during which increasing, doubling concentrations of a stimulus are administered by inhalation, each step being followed by measurement of the forced expiratory volume in 1 second (FEV\textsubscript{1}). A 20% fall in FEV\textsubscript{1} after administering a concentration of a stimulus (PC\textsubscript{20}) less than a certain threshold concentration, which differs for each stimulus inhaled, indicates the presence of AHR. Traditionally, two types of stimuli are being used for provocation testing: direct and indirect stimuli. Direct stimuli, such as histamine and methacholine (an acetylcholine analogue), act directly on the airway smooth muscle\cite{13}. Indirect stimuli, such as adenosine, act through adenosine receptors on inflammatory and neuronal cells which release mediators (e.g. histamine, leukotrienes and acetylcholine) upon stimulation leading to airway constriction\cite{14}. Of interest, the PC\textsubscript{20} adenosine 5’ monophosphate (AMP) is more strongly associated with eosinophilic airway inflammation than the PC\textsubscript{20} methacholine\cite{15}. This indicates that provocation testing with AMP can serve as a surrogate marker of airway inflammation. This hypothesis is strengthened by the observation that improvement in PC\textsubscript{20} AMP after treatment with inhaled corticosteroids, is closely related to decreased eosinophilic airway inflammation\cite{16}. A drawback of an AMP provocation test is that a substantial proportion of asthma patients does not reach a 20% fall in FEV\textsubscript{1} in response to the maximum concentration of AMP that can be nebulized\cite{17,18}. To address this disadvantage, our group has developed a dry powder adenosine formulation, which enables us to administer higher doses of inhaled adenosine. In two previous studies in asthma, the adenosine dry powder provocation test was shown to feasible, safe and well-tolerated\cite{19,20}.

**Small airways dysfunction**

*Prevalence of small airways dysfunction in asthma*

Small airways are defined as airways with an internal diameter less than 2mm generally starting at the 8\textsuperscript{th} generation of the bronchial tree. As small airways only account for a maximum of 15% of total airway resistance in the normal situation\cite{21}, the importance of small airways dysfunction in respiratory diseases has been ignored for decades. Nowadays, small airways are increasingly recognized as important contributors to clinical features of asthma, such as asthma control, frequency of exacerbations and nocturnal symptoms\cite{22}. Additionally, small airways dysfunction contributes to the severity of airway hyperresponsiveness. This was among others demonstrated by Telenga et al, who showed that more severe small airway obstruction, indicated by a lower forced expiratory flow at 50% (FEF\textsubscript{50}) of the forced vital capacity (FVC), was significantly associated with more severe hyperresponsiveness in asthma patients, independently from the FEV\textsubscript{1}\cite{23}. Also, Verbanck et al demonstrated that ventilation heterogeneity of the small conducting airways (S\textsubscript{cond}) was a major determinant of
airway hyperresponsiveness in asthma, independently of airway inflammation(24). The prevalence of small airways dysfunction in asthma is estimated to be 50-60% and can be present across all degrees of asthma severity, even in those patients with a normal FEV₁(25). Table 1 provides an overview of the prevalence of small airways dysfunction in asthma according to multiple measurement techniques.

**Measurements of small airways dysfunction**

Several techniques to measure small airways dysfunction are currently available, but, unfortunately, a golden standard has not yet been established. Traditionally, forced expiratory flow rates at 50% or between 25 and 75% of the FVC provide information on airflow obstruction originating from the small airways. In addition, Slow Vital Capacity (SVC) minus FVC or the FVC/SVC ratio reflects obstruction of the small airways during a forced expiratory maneuver. Hyperinflation is a result of small airways obstruction and can be measured with body plethysmography that provides information on residual volume (RV), functional residual capacity (FRC) and RV/total lung capacity (RV/TLC). Airway resistance and reactance can be measured with Impulse Oscillometry (IOS), as reflected by small airway resistance (R₅-R₂₀) or reactance (X₅). In addition, ventilation heterogeneity of the small conducting (Scond) and acinar (Sacın) airways is measured with multiple breath nitrogen washout (MBNW). Finally, air trapping as a result of small airways obstruction can be quantified on computed tomography (CT) – scans by comparing in- and expiratory density of the lung parenchyma: decreased expiratory attenuation of the lung parenchyma indicates air trapping.

**Treatment of asthma: extrafine and non-extrafine inhaled corticosteroids**

Inhaled corticosteroids (ICS) have been the mainstay of asthma treatment since the introduction of beclomethasone dipropionate in pressurized aerosols in the 1970s. Years of extensive research have taught us that ICS reduce airway inflammation and improve airway hyperresponsiveness to both direct and indirect stimuli in asthma patients(16,28–30). Ward and colleagues showed that 12-month treatment with fluticasone significantly decreases airway hyperresponsiveness compared to placebo, with a maximum effect after continued treatment, in this case 12 months(30). Of interest, they showed that the number of eosinophils and mast cells in bronchoalveolar lavage (BAL) fluid significantly decreases after 3-month treatment with fluticasone compared to placebo, with no further changes after 12-month treatment. Thus, ICS continuously decrease airway hyperresponsiveness, while the maximal diminishing effect of ICS on airway inflammation is reached after 3 months. This suggests that other beneficial effects of ICS, next to a reduction in inflammation, contribute to the ongoing improvement in airway hyperresponsiveness in this population.
### Table 1. Prevalence of small airways dysfunction in asthma

<table>
<thead>
<tr>
<th>Small airways measurement</th>
<th>Cut-off for SAD</th>
<th>Prevalence</th>
<th>Characteristics asthma population</th>
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<tr>
<td><strong>Spirometry</strong></td>
<td></td>
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<tr>
<td>FEF&lt;sub&gt;25-75%&lt;/sub&gt;</td>
<td>&lt; 60% predicted</td>
<td>54%</td>
<td>442 asthma patients (step 1-4 BTS asthma guidelines)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>&lt; LLN</td>
<td>12%</td>
<td>222 asthma patients without proximal airways obstruction&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>FEF&lt;sub&gt;50%&lt;/sub&gt;</td>
<td>&lt; LLN</td>
<td>36%</td>
<td>94 mild-to-moderate asthma patients</td>
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<td>SVC-FVC</td>
<td>&gt; 10%</td>
<td>10%</td>
<td>222 asthma patients without proximal airways obstruction&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Body Plethysmography</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV</td>
<td>&gt; 100% predicted</td>
<td>52%</td>
<td>321 predominantly mild asthma patients&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>&gt; ULN</td>
<td>31%</td>
<td>222 asthma patients without proximal airways obstruction&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>RV/TLC</td>
<td>&gt; 35%</td>
<td>57%</td>
<td>321 predominantly mild asthma patients&lt;sup&gt;c&lt;/sup&gt;</td>
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<td></td>
<td>&gt; ULN</td>
<td>24%</td>
<td>222 asthma patients without proximal airways obstruction&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>&gt; ULN</td>
<td>48%</td>
<td>305 poorly controlled asthma patients&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>FRC</td>
<td>&gt; 120% predicted</td>
<td>26%</td>
<td>222 asthma patients without proximal airways obstruction&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>&gt; 120% predicted</td>
<td>48%</td>
<td>305 poorly controlled asthma patients&lt;sup&gt;d&lt;/sup&gt;</td>
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<td><strong>Impulse Oscilometry</strong></td>
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<td>R&lt;sub&gt;5&lt;/sub&gt;-R&lt;sub&gt;20&lt;/sub&gt;</td>
<td>&gt; 0.03 kPa s L&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>48%</td>
<td>63 mild-to-moderate asthma patients</td>
</tr>
<tr>
<td></td>
<td>&gt; 0.03 kPa s L&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>65%</td>
<td>192 asthma patients (step 2 BTS asthma guidelines)&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>&gt; 0.03 kPa s L&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>64%</td>
<td>63 asthma patients (step 3 BTS asthma guidelines)&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>&gt; 0.03 kPa s L&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>70%</td>
<td>123 asthma patients (step 4 BTS asthma guidelines)&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>&gt; 0.075 kPa s L&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>33%</td>
<td>33 asthma patients with FEV&lt;sub&gt;1&lt;/sub&gt; ≥ 80% predicted</td>
</tr>
<tr>
<td></td>
<td>&gt; 0.1 kPa s L&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>42%</td>
<td>442 asthma patients (step 1-4 BTS asthma guidelines)&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Multiple Breath Nitrogen Washout</strong></td>
<td></td>
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<tr>
<td>S&lt;sub&gt;acin&lt;/sub&gt;</td>
<td>&gt; ULN*</td>
<td>46%</td>
<td>37 asthma patients (step 3-5 GINA guidelines)</td>
</tr>
<tr>
<td></td>
<td>&gt; 0.12 L&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>53%</td>
<td>66 well-controlled asthma patients</td>
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<tr>
<td></td>
<td>&gt; 0.12 L&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>53%</td>
<td>30 stable unselected asthma patients&lt;sup&gt;**&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>&gt; ULN**</td>
<td>61%</td>
<td>18 asthma patients admitted for severe exacerbation&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>&gt; ULN**</td>
<td>74%</td>
<td>19 stable asthma patients&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>S&lt;sub&gt;cond&lt;/sub&gt;</td>
<td>&gt; 0.042</td>
<td>100%</td>
<td>30 stable unselected asthma patients&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>&gt; ULN**</td>
<td>56%</td>
<td>18 asthma patients admitted for a severe exacerbation&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>&gt; ULN**</td>
<td>11%</td>
<td>19 stable asthma patients&lt;sup&gt;1&lt;/sup&gt;</td>
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<td><strong>CT scan</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Air trapping</td>
<td>Air trapping index&lt;sup&gt;***&lt;/sup&gt;</td>
<td>56%</td>
<td>45 uncontrolled mild or moderate asthma patients</td>
</tr>
</tbody>
</table>

Prevalences adapted from Usmani et al (25); an equal symbol indicates the same study; *as defined as mean +1.64SD in the control group; **adapted from Verbanck et al (26); ***according to the definition of the Fleischner Society (27); †defined as FEV<sub>1</sub>% predicted > 80% and FEV<sub>1</sub>/FVC < 0.7; SAD = small airways dysfunction; LLN = lower limit of normal; ULN = upper limit of normal; BTS = British Thoracic Society; GINA = Global Initiative for Asthma; FEF<sub>25-75%</sub> = forced expiratory flow between 25 and 75% of the FVC; FEF<sub>50%</sub> = forced expiratory flow at 50% of the FVC; SVC = slow vital capacity; FVC = forced vital capacity; RV = residual volume; TLC = total lung capacity; FRC = functional residual capacity; R<sub>5</sub>-R<sub>20</sub> : resistance between 5 and 20Hz; S<sub>acin</sub> = ventilation heterogeneity of the acinar airways; S<sub>cond</sub> = ventilation heterogeneity of the conducting airways.
Additionally, ICS effectively reduce respiratory symptoms, improve asthma control and protect against exacerbations and asthma-related mortality(31–33). The latter was investigated in a nested case-control study of 66 subjects who died of asthma and 2681 matched asthma controls(31). The authors concluded that subjects who died of asthma had used significantly less canisters of low-dose ICS compared to their matched controls, irrespective of age, gender, prior hospitalization for asthma and the use of other asthma medication. In conclusion, these findings underline the beneficial effects of ICS in the treatment of asthma.

**Extrafine and non-extrafine particle ICS**

Extrafine particle ICS have a mass median aerodynamic diameter (MMAD) smaller than 2 µm, in contrast to non-extrafine particle ICS, which have a MMAD between 2 and 4 µm(34,35). It has been shown that peripheral lung deposition is higher after administration of monodisperse particles sized 1.5 µm (25%) compared to particles sized 3 µm (17%) and 6 µm (11%)(36). In the latter study, lower oropharyngeal deposition was observed for 1.5 µm-sized particles (15%) compared to particles with an MMAD of 3 µm (31%) and 6 µm (43%). These results confirm the hypothesis that higher peripheral lung deposition and lower oropharyngeal deposition is achieved with extrafine particle ICS. Many, but not all, clinical studies comparing extrafine and non-extrafine particle ICS show beneficial effects of extrafine particle treatment in terms of asthma control, improvement of small airways function and hospitalization(37–40). For example, Yamaguchi et al showed that asthma patients treated for 12 weeks with extrafine HFA-beclomethasone 200 µg b.i.d. (n=26) had a significant higher decrease in $R_{5-20}$ compared to asthma patients treated for 12 weeks with non-extrafine CFC-beclomethasone 400 µg b.i.d. (n=12) (37). Of interest, no significant difference in the change of FEV$_1$ was observed between the two treatments. In addition, Hoshino et al showed that 14 asthma patients randomly assigned to receive extrafine ciclesonide 200 µg q.d. for 8 weeks had a significant improvement in small airways function as measured with $R_{5-20}$ and $X_{5}$ while small airways function in 16 asthma patients receiving non-extrafine fluticasone 100 µg b.i.d. for 8 weeks remained unchanged(38). Again, FEV$_1$ was not affected by either extrafine ciclesonide or non-extrafine fluticasone in this study. Finally, a real-life comparison study based on a retrospective cohort of ~20,000 primary care asthma patients compared treatment with extrafine HFA-beclomethasone (Qvar) and non-extrafine HFA-beclomethasone (Clenil). The authors show that better asthma control was achieved with Qvar, as well as less respiratory-related hospitalizations(39). These findings further support the notion that extrafine particle ICS are beneficial for asthma patients, when compared to non-extrafine particle ICS. However, large double-blind prospective studies that compare the same drugs in the same devices with the
only difference being particle size are lacking to confirm these observations. In addition to this, it could be speculated that certain subgroups of asthma patients would benefit in particular from extrafine ICS treatment, but studies addressing this need, i.e. the identification of a so-called ‘extrafine particle sensitive phenotype’, are not available. Future studies are necessary to explore which asthma phenotype specifically benefits from extrafine or non-extrafine ICS.

**Remission of asthma**

Currently, no cure for asthma exists. Of interest, a considerable number of asthma patients experiences ‘clinical remission’, which is defined as the absence of respiratory symptoms and no use of asthma medication at a certain moment in life. It is estimated that clinical remission rates vary between 30% and 52%(41). A decade ago, the term ‘complete remission’ was introduced, which specifies remission by adding extra criteria to the definition: a normalized pulmonary function and absence of airway hyperresponsiveness. Vonk et al applied this new definition to assess remission rates in a cohort of 119 allergic asthmatic children with a mean follow-up of 30 years, i.e. up to adult life(42). In total, 52% of subjects experienced remission of which 22% were in complete remission and 30% were in clinical remission. In another study, 209 asthmatic children with proven airway hyperresponsiveness were followed-up at age 25 and 49 years(43). The prevalence of complete remission was 7% at age 25 and 10% at age 49. These studies emphasize that remission of asthma occurs in a small percentage, but such cases still represent a substantial number of asthma patients. Knowledge on underlying mechanisms that drive asthma remission can aid in developing new asthma therapies that might even lead to a cure of asthma. In this respect, especially the phenotype complete remission is of interest. This phenotype comprises patients with a normalized pulmonary function and absence of hyperresponsiveness, despite not using any asthma treatment, which is suggestive of a reversible biological factor driving remission of asthma. On the other hand, subjects in clinical remission still exhibit an aberrant pulmonary function, which raises the question whether biological processes or an altered perception of symptoms play a role in this population.

Studies investigating the underlying pathophysiology of complete asthma remission mechanisms are scarce. Broekema et al studied subjects with either current asthma, clinical remission or complete remission(44). They showed that the number of sputum eosinophils was not statistically different between the groups but eosinophilic activation in the airway wall, measured with eosinophilic peroxidase (EPX) staining, was significantly lower in complete remission versus both clinical remission and current asthma (Figure 1A-B). Of interest, basement membrane thickening, a feature
of airway remodeling, was similar between complete remission and current asthma (Figure 1C). Boulet et al investigated subjects in complete remission, clinical remission and current asthma\(^{45}\). Their findings support the observations of Broekema et al in that inflammatory cells in sputum are similar across the three groups. They additionally showed that the suppressive function of regulatory T-cells is decreased in both complete remission and asthma compared to healthy controls. These findings raise the hypothesis that an altered activation of eosinophilic inflammation, but not necessarily reversed remodeling or normalization of regulatory T-cell function, contribute to complete remission of asthma.

Figure 1. Eosinophilic inflammation and basement membrane thickening in complete remission, clinical remission, asthma patients not using inhaled corticosteroids (no ICS) and asthma patients using inhaled corticosteroids (ICS) (reprinted with permission from reference 44); A) percentage of sputum eosinophils, B) eosinophilic peroxidase (EPX) immunopositivity (pixels) in bronchial biopsies and C) basement membrane thickness. P-values are adjusted for age, sex and current smoking.
Chapter 1

Smoking and respiratory health

Tobacco smoking accounts for more than 6 million deaths per year, according to the World Health Organization(46). It is a major cause of respiratory diseases, such as Chronic Obstructive Pulmonary Disease (COPD) and lung cancer. Next to this, smoking negatively influences the clinical expression of asthma(47). In addition, even in subjects without respiratory diseases smoking is associated with increased airway inflammation and more small airways dysfunction(48,49).

COPD

The effects of smoking on the respiratory tract that contribute to the development of COPD are numerous(50,51). First, smoking decreases the integrity of the respiratory epithelium and impairs cilia function, causing a diminished barrier function leading to an impaired defense against toxins and microorganisms. Second, airway inflammation is enhanced by smoking through proliferation of macrophages in the alveolar space, which produce pro-inflammatory cytokines leading to recruitment of neutrophils, eosinophils and lymphocytes. Also, the phagocytic properties of macrophages are impaired, which causes accumulation of dead cells and, subsequently, increased inflammation. Of importance, macrophages produce reactive oxygen species and proteases, which induce tissue destruction leading to emphysema. Third, cigarette smoke also has immunosuppressive effects, amongst others illustrated by less airway natural-killer cells and inhibition of the memory T-cell response in smokers compared to non-smokers, leading to an increased susceptibility for respiratory infections. Of interest, the majority of smokers does not develop COPD, which implies that next to exposure to noxious particles, other factors such as genetic susceptibility are of great importance. It remains a major challenge to identify the so-called 'susceptible smoker', i.e. those smokers that will develop COPD upon exposure to risk factors.

Asthma and smoking

Surprisingly, smoking is as prevalent among asthma patients as in the general population(52). Asthma patients who smoke experience more respiratory symptoms and have more frequent exacerbations(53). Furthermore, smoking is associated with an accelerated lung function decline in asthma patients(54,55). In a population study of 234 subjects with asthma performed in Denmark, Lange et al found heavy smokers (15g tobacco/day) to have an additional decline in FEV₁ of 22.5ml/year compared to non-smokers(54). These findings were confirmed by O'Byrne et al, who showed that the 3-year decline in FEV₁ in 492 asthmatic smokers and 2432 asthmatic non-smokers was 264ml versus 181mL, respectively(55). Of interest, smokers with asthma show a
diminished response to inhaled corticosteroids, although clinical trials addressing ICS treatment specifically in asthmatic smokers are limited(55–59). Telenga et al showed that current smokers (n=30) with asthma had a lower increase in FEV\textsubscript{1} compared to never-smokers (n=55) after 2-week ICS treatment, although this effect was not observed after 1-year treatment(56). Short-term effects of ICS were also studied by Chalmers et al, comparing 3-week treatment with 1000µg fluticasone daily in smokers (n=17) and never-smokers (n=21) with asthma(57). They showed that in never-smokers, ICS decreased peak expiratory flow (PEF) variability, increased FEV\textsubscript{1} and improved bronchial hyperresponsiveness while in smokers, no significant change in any of these parameters was observed. Long-term studies on ICS effects are scarce. Pederson et al showed that 9-month treatment with either high (1600µg/d) or low (400µg/d) dose budesonide did not improve FEV\textsubscript{1} or hyperresponsiveness in asthmatic smokers (n=37), while asthmatic never- and ex-smokers (n=47) did significantly improve in both parameters(58). On the other hand, in a large randomized trial with 3-year follow-up in which asthma patients were randomized between 400µg budesonide daily and placebo, smokers (n=492) and non-smokers (n=2432) were equally responsive to budesonide in terms of decline in FEV\textsubscript{1}(55). Tomlinson et al treated 28 non-smokers and 19 smokers with asthma for 12 weeks with 400µg beclomethasone daily and showed a significant decrease in PEF variability after treatment in non-smokers, when compared to smokers(59). However, after 12-week treatment with a higher dose of ICS, i.e. 1200µg daily, no difference in change in PEF variability between smokers (n=21) and non-smokers (n=27) was observed.

Findings from these studies emphasize that: 1) studies on ICS treatment in smokers with asthma are scarce, 2) study outcomes in individual studies (i.e. PEF variability, FEV\textsubscript{1}, hyperresponsiveness) are variable, which hampers the direct comparison of these studies, 3) short term effects of ICS seems to be impaired in smokers with asthma but 4) longer treatment duration or higher doses of ICS might be more effective. Based on these findings, the exact role of ICS in asthma patients who smoke is not exactly clear. In conclusion, smoking is prevalent in asthma and contributes to more severe disease. Although smoking cessation is essential for these patients, achieving this appears difficult. Studies targeting this specific group of asthma patients are needed to improve treatment and eventually clinical outcome.

**Small airways dysfunction and smoking**

Smoking is known to induce small airways dysfunction. The latter was demonstrated by Verbanck et al, who showed that smokers with more than 10 pack-years showed increased ventilation heterogeneity (i.e. higher $S_{\text{cond}}$ and $S_{\text{acin}}$) of the small airways compared to never-smokers(48). These findings can potentially be explained by higher numbers of inflammatory cells and higher levels of inflammatory mediators present in
the small airways of smokers compared to non-smokers\((60,61)\), which in turn may lead to persistent airway remodeling and narrowing. As mentioned previously, the majority of studies investigating ICS-treatment in smokers with asthma have used non-extrafine particle ICS, which mainly deposit in the central airways. It could be speculated that extrafine particle ICS, which show a higher preference for deposition in the peripheral airways, are more beneficial in smokers with asthma.

**MOLECULAR PHENOTYPING**

**Genomic research in obstructive lung diseases**

In the past decades, new techniques have become available to study underlying pathophysiological mechanisms of obstructive lung diseases, such as genome wide association (GWA) studies and transcriptomics. GWA studies have identified genomic regions containing DNA variants that possibly play a role in asthma development. These genomic regions contain genes such as \textit{SMAD3} and \textit{ORMDL3} for asthma\((62)\) and \textit{HHIP} and \textit{FAM13A} for COPD\((63)\). Transcriptomics is defined as ‘the study of the transcriptome’, which comprises the complete set of RNA transcripts present in a cell, varying from protein-coding messenger RNA transcripts to non-coding RNA transcripts such as microRNAs and long non-coding RNAs. Analysis of RNA expression levels in obstructive lung diseases is nowadays a topic of wide interest and can aid in understanding mechanisms driving a disease. For example, a study investigating the bronchial epithelium in COPD has shown that COPD patients exhibit a distinct gene expression profile compared to controls which resembles COPD-induced gene expression changes occurring in lung tissue\((64)\). In addition, bronchial epithelial gene expression in COPD changes after treatment with ICS and this altered gene expression profile is enriched in genes associated with smokers without COPD versus smokers with COPD, suggesting that ICS can reverse disease activity\((65)\). Results of these studies investigating the transcriptome in COPD are of interest in two ways: 1) they provide information on transcriptional mechanisms driving a disease or a treatment and 2) transcriptional alterations can potentially serve as a biomarker to aid in e.g. diagnosing a disease or monitoring response to treatment. In this respect, studies on bronchial gene expression in asthma are also of interest. Woodruff and colleagues found 22 genes to be differentially expressed in the airways of asthma patients compared to controls, of which 3 genes were also affected by 1-week ICS treatment: \textit{POSTN}, \textit{CLCA1} and \textit{SERPINB2}\((66)\). They additionally showed that higher baseline expression of these 3 genes correlated with improvement in FEV\(_1\). Further, a study comparing airway gene expression between patients with asthma and COPD, identified overlapping gene expression changes suggesting changes in biological processes that are shared between asthma and COPD\((67)\). These findings
are illustrative of the role of transcriptomics as a potential biomarker and as a tool to explore disease-mechanisms. Although transcriptional findings in the airway wall are of great interest, extensive research is hampered by the difficulty to obtain these samples, which requires an invasive bronchoscopy. Alternatives are currently explored, such as less invasive sampling from the nasal epithelium. It has been shown that the smoking-associated nasal epithelial gene expression profile shows considerable overlap with the smoking-associated bronchial gene expression profile(68,69). This implies that the nasal epithelium is a promising site for future transcriptomic research in asthma and COPD.

OUTLINE OF THE THESIS

In Chapter 2 we investigate subjects with proven airway hyperresponsiveness who do not experience any respiratory symptoms, so-called ‘asymptomatic airway hyperresponsiveness (AHR)’. Since small airways dysfunction is associated with respiratory symptoms and with AHR, we hypothesized that subjects with asymptomatic AHR have less small airways dysfunction than subjects with current asthma. We performed a cross-sectional study including subjects with asthma, subjects with asymptomatic AHR and healthy controls, in which we especially focused on small airways function measured with impulse oscillometry at baseline and during a methacholine provocation test.

Chapter 3 investigates the presence and extent of small airways dysfunction, emphysema and parenchymal disease measured with CT-scans in association with ageing and smoking in a healthy population. We applied a new technique to assess CT-scans, ‘parametric response mapping’, which is based on a voxel-by-voxel comparison of lung attenuation on in- and expiratory CT-scans, which has the advantage that it allows discrimination between emphysema and small airways dysfunction.

In Chapter 4 we describe results of the OLiVIA study: a randomized controlled trial in which we compared extrafine with non-extrafine particle ICS treatment in smokers and ex-smokers with asthma. Since smoking induces small airways dysfunction, we hypothesized that smokers and ex-smokers with asthma would benefit more from extrafine compared to non-extrafine ICS. In Chapter 5, we performed a post-hoc analysis of this study in which we investigated whether subjects having small airways dysfunction at baseline, benefit more from extrafine compared to non-extrafine ICS. We additionally explored which other clinical variables predict a favorable response to either extrafine or non-extrafine ICS.

Chapter 6 describes the comparison of two formulations of adenosine used for bronchial provocation testing in asthma: wet nebulized adenosine 5’ monophosphate (AMP) and the recently developed dry powder adenosine. Since not all asthma patients experience a 20% fall in FEV₁ after administering the highest possible dose of AMP, and dry powder
adenosine can be produced in higher doses, we hypothesized that dry powder adenosine would be a more sensitive test to measure airway hyperresponsiveness in asthma.

In Chapter 7 we aimed to define a nasal epithelial gene expression profile that distinguishes smoking COPD patients from smoking controls. We additionally explored whether this gene expression profile was overlapping with COPD-associated gene expression in the bronchial epithelium in two independent cohorts. 

Chapter 8 describes our investigations to assess whether ICS treatment in smokers and ex-smokers with asthma is reflected by changes in nasal epithelial gene expression in 2 cohorts of asthma patients treated for 2 weeks and 3 months with ICS. We furthermore explored whether these ICS-induced nasal gene expression changes overlap with corticosteroid-induced bronchial gene expression changes in 2 independent cohorts of asthma patients and in air-liquid interface cultures of human bronchial epithelial cells. 

Chapter 9 describes a study in which we aim to unravel underlying pathophysiological mechanisms driving remission of asthma by analysis of microRNA expression in bronchial biopsies of subjects with complete remission of asthma, subjects with persistent asthma and healthy controls. We integrated microRNA expression with protein-coding- and long non-coding RNA expression by performing Bayesian network modeling, with the aim to gain insight in the complex interactions of transcripts contributing to remission of asthma.
REFERENCES


Chapter 1


General introduction


Chapter 1


PART I

Clinical Phenotyping