Summary

Lung cancer and chronic obstructive pulmonary disease (COPD) are two smoking-related respiratory disorders that are both leading causes of mortality worldwide. Between 50% and 90% of smokers diagnosed with lung cancer have pre-existing COPD. The frequent co-occurrence of both diseases in the same patient is not only due to shared risk factors, such as age and smoking, but also because COPD independently increases the risk of lung cancer up to fivefold. COPD is a complex disease that is characterized by progressive lung function decline and an abnormal immune response in the lungs. The severity of disease is determined by the degree of airflow limitation, caused by small airway obstruction and parenchymal destruction (emphysema). Epidemiological data indicate that both components independently predict the incidence of lung cancer. Furthermore, COPD and lung cancer are also linked in never-smokers. The close association between both diseases suggests that there are shared risk factors other than cigarette smoking, or that abnormalities in COPD lungs influence the development of cancer. However, the precise mechanisms involved remain poorly understood.

While smoking accounts for over 80% of all COPD and lung cancer cases, only a minority (<25%) of smokers will develop one or both of the diseases. This observation suggests an inherited or genetic difference in the response to cigarette smoke among smokers. Moreover, the fact that the same minority of smokers that is susceptible to develop COPD seems also to be susceptible to develop lung cancer suggests the presence of a shared genetic susceptibly to both diseases. Overlapping susceptibility variants have indeed been reported. In particular, the majority of genome-wide association studies (GWAS) on COPD and on lung cancer identified the chromosome 15q24/25 locus that contains (among other genes) the *CHRNA* genes encoding nicotinic acetylcholine receptor subunits. However, the same *CHRNA* variants that associated with COPD and lung cancer were also discovered as genetic predictors of smoking behavior, making it more difficult to distinguish between a direct genetic association with disease and an indirect association via an influence on smoke exposure. In contrast, the second locus associated with lung cancer – the chromosome 5p15.33 locus that contains *TERT* and *CLPTM1L* - is thought to associate directly with lung cancer. Replication of the study in never-smokers confirmed a direct association, while a GWAS on smoking addiction showed no such association. At the time we initiated our studies,

the relationship of 5p15.33 with COPD and emphysema had not yet been investigated.

In this thesis, we explored the hypothesis of a common genetic basis for COPD, emphysema and lung cancer by comparing genotype frequencies between smokers with no respiratory disease, smokers with COPD in whom emphysema was radiographically assessed, and smokers with lung cancer in whom spirometry had been performed to identify coexisting COPD. We also explored the role of the DNA methylome in the link between COPD and lung cancer. DNA methylation is an important regulator of gene expression and is susceptible to modification by the environment. Given the importance of the environment to the development of COPD and lung cancer, the DNA methylome is likely to play a role in both diseases, and potentially also in the link between them. Alterations in the normal DNA methylation pattern have indeed been described in peripheral blood, bronchial airway epithelial cells and tissue samples of COPD and lung cancer patients. While there seemed to be an overlap between the differential methylation patterns found in both diseases, the direct influence of COPD on the lung cancer methylome had not yet been investigated. To study this influence, we prospectively collected tumor and adjacent non-malignant lung tissue of non-small cell lung cancer (NSCLC) patients that were all carefully phenotyped for smoking behavior, COPD status and the presence of emphysema.

This unique approach led to the identification of rs31489 in the *TERT-CLPTM1L* locus as a shared and independent susceptibility factor for both COPD, emphysema and lung cancer. In addition, carriers of the at-risk genotype had a twofold higher risk of developing both lung cancer and COPD than lung cancer alone. We also found that *CHRNA* variants associate with accelerated lung function decline in asymptomatic current smokers. We did not identify an association between *CHRNA* variants and smoking-related variables per se. However, the effect on lung function decline was only present in current smokers (and not in former smokers), suggesting both a direct influence potentially through affecting nicotinic acetylcholine receptors expressed on airway epithelial cells and immune cells, and an indirect effect mediated by differences in smoking behavior according to *CHRNA* variants. In addition, we identified a significant association between the same *CHRNA* variants and relevant COPD outcomes, *i.e.* an increased risk of developing very severe COPD and of evolving to end-stage COPD with need of lung transplantation.

By use of unbiased DNA methylation profiling, we could establish that the presence of COPD influences the methylation profile of lung tumors. Ontology analysis of differentially methylated genes revealed a strong enrichment of immune genes in COPD tumors, but not in non-COPD tumors. This COPD-associated immune signature was attributable to methylation changes in immune genes expressed either by tumor cells or tumor-infiltrating immune cells. No such differences were observed in the adjacent non-malignant tissue. Subsequent transcriptome profiling confirmed that genes involved in the immune response were differentially expressed in COPD tumors, an observation that was independently replicated using publicly available data from lung cancer patients in The Cancer Genome Atlas (TCGA). Finally, immunohistochemistry validated these findings, revealing fewer CD3- and CD4-positive T lymphocytes in tumors derived from patients with COPD. Overall, we thus identified a NSCLC subtype characterized by reduced immune cell infiltration and associated with COPD status. These findings may seem surprising since COPD lungs show immune cell infiltration and excessive chronic inflammation. However, dysfunctional immune reactions have been established in COPD lungs, which may explain a reduced anti-tumor immune response. Hence, our data seem biologically plausible. In addition, especially in light of the relentless search for predictive biomarkers for novel immunotherapies, the identification of a reduced anti-tumor immune response in COPD patients seems also clinically relevant. We therefore suggest that future studies assessing novel NSCLC treatments should take COPD status or reduced immune cell infiltration as a potential predictive biomarker into account.

Overall these results show that genetic and epigenetic association studies can contribute to a better understanding of the link between COPD and lung cancer by unraveling the pathways involved. In addition, results may be relevant for risk prediction and the optimization of novel therapies.