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CLINICAL AND GENETIC MARKERS IN DISEASE PROGRESSION – A STUDY AMONG SUBJECTS WITH OBSTRUCTIVE LUNG DISEASES

Henna Kupiainen

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ABSTRACT

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Clinical and genetic markers in disease progression – a study among subjects with obstructive lung diseases

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Asthma, COPD, and asthma and COPD overlap syndrome (ACOS) are chronic pulmonary diseases with an obstructive component. In COPD, the obstruction is irreversible and the disease is progressive.

The aim of the study was to define and analyze factors that affected disease progression and patients' well-being, prognosis and mortality in Chronic Airway Disease (CAD) cohort. The main focus was on COPD and ACOS patients. Retrospective data from medical records was combined with genetic and prospective follow-up data.

Smoking is the biggest risk factor for COPD and even after the diagnosis of the disease, smoking plays an important role in disease development and patient's prognosis. Sixty percent of the COPD patients had succeeded in smoking cessation. Patients who had managed to quit smoking had lower mortality rates and less psychiatric diseases and alcohol abuse although they were older and had more cardiovascular diseases than patients who continued smoking. Genetic polymorphism rs1051730 in the nicotinic acethylcholine receptor gene (*CHRNA3/5*) associated with heavy smoking, cancer prevalence and mortality in two Finnish independent cohorts consisting of COPD patients and male smokers. Challenges in smoking cessation and higher mortality rates may be partly due to individual patient's genetic composition.

Approximately 50% of COPD patients are physically inactive and the proportion was higher among current smokers. Physically active and inactive patients didn't differ from each other in regard to age, gender or comorbidities. Bronchial obstruction explained inactivity only in severe disease. Subjective sensation of dyspnea, however, had very strong association to inactivity and was also associated to low health related quality of life (HRQoL). ACOS patients had a significantly lower HRQoL than either the patients with asthma or with COPD even though they were younger than COPD patients, had better lung functions and smaller tobacco exposure.

Keywords: COPD, asthma, ACOS, smoking cessation, HRQoL, physical activity, dyspnea, mortality, genetic polymorphism, retrospective study, prospective study.

TIIVISTELMÄ

Henna Kupiainen

Kroonisten obstruktiivisten keuhkosairauksien etenemisen ennustaminen kliinisten ja geneettisten muuttujien avulla

Turun yliopisto, Lääketieteellinen tiedekunta, Kliininen laitos, Keuhkosairausoppi ja kliininen allergologia, Turun yliopiston kliininen tohtoriohjelma (TKT); Keuhkosairauksien tutkimusyksikkö, Helsingin yliopistollinen keskussairaala, Helsinki, Suomi.
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Astma, keuhkoahtaumatauti ja sekamuotoinen astma-COPD (ACOS) ovat kroonisia, obstruktiivisia keuhkosairauksia. Keuhkoahtaumataudissa obstruktio on pysyvä, ja tauti luonteeltaan etenevä. Tutkimuksen tavoitteena oli tutkia tekijöitä, jotka vaikuttavat taudin etenemisnopeuteen, potilaiden vointiin ja ennusteeseen Chronic Airway Disease (CAD) –kohortin potilailla. Tutkimus keskittyi erityisesti keuhkoahtaumatautipotilaisiin. Sairauskertomusten retrospektiivista dataa pyrittiin hyödyntämään yhdessä geneettisten markkereiden ja seurantakyselyistä kerätyn prospektiivisen tiedon kanssa.

Tupakointi on suurin yksittäinen keuhkoahtaumataudin riskitekijä ja taudin puhjettuakin tupakoinnin määrä vaikuttaa potilaan ennusteeseen. 60% keuhkoahtaumatautipotilaista onnistui lopettamaan tupakoinnin. Lopettaneiden kuolleisuus oli pienempää ja heillä esiintyi vähemmän psykiatrisia sairauksia ja alkoholismia kuin tupakointia jatkavilla, vaikka lopettaneet olivatkin vanhempia ja heillä esiintyi enemmän sydän- ja verisuonisairauksia. Nikotiiniherkän asetyylikoliinireseptorigeenin (CHRNA3/5) polymorfia rs1051730 assosioitui pitkäkestoiseen tupakointiin, syöpäprevalenssiin ja kuolleisuuteen kahdessa erillisessä suomalaiskohortissa, joista toinen koostui keuhkoahtaumatautipotilaista ja toinen tupakoivista miehistä. Erot potilaiden genotyypissä voivat osaltaan vaikuttaa tupakoinnin lopettamiseen ja kuolleisuuteen.

Noin 50 % kohortin keuhkoahtaumatautipotilaista oli fyysisesti inaktiivisia. Fyysisesti aktiivisten ja inaktiivisten potilaiden i'issä, sukupuolijakaumassa tai liitännäis-sairauksissa ei havaittu eroja. Obstruktio selitti inaktiivisuutta vasta kun tauti oli vaikea. Sen sijaan subjektiivinen hengenahdistuksen tunne selitti inaktiivisuutta voimakkaasti jo lievässä taudissa. ACOS-potilaiden terveyteen liittyvä elämänlaatunsa oli merkittävästi huonompi kuin astma- tai keuhkoahtaumatautipotilailla.

Avainsanat: keuhkoahtaumatauti, astma, sekamuotoinen astma-COPD, tupakoinnin lopettaminen, terveyteen liittyvä elämänlaatu, fyysinen aktiivisuus, hengenahdistus, kuolleisuus, geneettinen polymorfia, retrospektiivinen tutkimus, prospektiivinen tutkimus

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ABBREVIATIONS

ACOS	asthma COPD overlap syndrome
AQ20	Airway questionnaire 20
BMI	body mass index
BODE index	BMI, Obstruction, Dyspnea and Exercise capacity index
CAD cohort	Chronic Airway Disease cohort
CHRNA5/3	nicotinic acetylcholine receptor gene locus
CI	confidence interval
COPD	chronic obstructive pulmonary disease
COPEX	COPD Exercise study
CVD	cardiovascular diseases (coronary artery disease, cerebrovascular disease, peripheral arterial disease)
DLCOcVA	diffusing capacity for carbon monoxide, corrected for alveolar volume and hematocrit value
FEV1	forced expiratory volume in 1 second
FEV1 of pred	percent of FEV1 from predicted value
FEV%	FEV1/FVC x 100 (%)
FTND	Fagerström test for nicotine dependence
FVC	forced vital capacity
GINA	Global Initiative for Asthma
GOLD	Global Initiative for Chronic Obstructive Lung Disease
GOLD criteria	GOLD criteria for COPD diagnosis and classification
GWAS	genome wide association study
HR	hazard ratio

HRQoL	health related quality of life
HUCH	Helsinki University Central Hospital
MAF	minor allele frequency
MMRC dyspnea scale	Modified (British) Medical Research Council dyspnea scale
ND	nicotine dependency
NRT	nicotine replacement therapy
OR	odds ratio
PEF	peak expiratory flow
QoL	quality of life
SD	standard deviation
SNP	single nucleotide polymorphism
SPSS	Statistical Package for Social Sciences
TUCH	Turku University Central Hospital
15D	15 dimensional quality of life questionnaire
6MWD test	6 minutes walking distance test

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by the Roman numerals I–IV, and on some supplementary unpublished data.

I Kauppi P, Kupiainen H, Lindqvist A, Tammilehto L, Kilpeläinen M, Kinnula VL, Haahtela T, Laitinen T. Overlap syndrome of asthma and COPD predicts low quality of life. J Asthma. 2011 Apr;48(3):279-85.

II Katajisto M, Kupiainen H, Rantanen P, Lindqvist A, Kilpeläinen M, Tikkanen H, Laitinen T. Physical inactivity in COPD and increased perception of dyspnea. Int J Chron Obstruct Pulmon Dis. 2012:7:743-55.

III Kupiainen H, Kinnula VL, Lindqvist A, Postma DS, Boezen HM, Laitinen T, Kilpeläinen M. Successful Smoking Cessation in COPD: Association with Comorbidities and Mortality. Pulm Med. 2012;2012:725024.

IV Kupiainen H, Kuokkanen M, Kontto J, Virtamo J, Salomaa V, Lindqvist A, Kilpeläinen M, Laitinen T. CHRNA5/CHRNA3 locus associates with increased mortality among smokers.

Accepted for publication in: COPD: Journal of Chronic Obstructive Pulmonary Disease.

1. INTRODUCTION

Asthma and chronic obstructive pulmonary disease (COPD) are chronic airway diseases. In asthma, the obstruction is typically reversible, whereas COPD is characterized by mainly irreversible obstruction of bronchioles and destruction of lung parenchyma leading to emphysema. Asthma and COPD overlap syndrome (ACOS) is a new definition for patients whose symptoms and clinical findings cover those of asthma and COPD and fill the diagnostic criteria of both the diseases. (GINA-GOLD. Diagnosis of diseases of chronic airflow limitation: asthma, COPD and ACOS.) In Finland asthma prevalence has been estimated to be 10% and COPD prevalence 5–9% in middle-aged and elderly people. (Vasankari, Impivaara et al. 2010, Kainu, Pallasaho et al. 2013) In Finland the prevalence of ACOS patients has been estimated to be 16–27% among diagnosed asthma and COPD patients. (Andersen, Lampela et al. 2013, Kiljander, Helin et al. 2015)

The main risk factor for COPD in developed countries is smoking. Toxic compounds in tobacco smoke trigger and maintain inflammation in the lungs, which results in typical pathophysiological changes and symptoms of COPD. COPD patients display also signs of systemic inflammation, especially in severe stages of the disease and during exacerbations. The most common symptoms are cough, sputum production and dyspnea upon exertion. It has been estimated that 20–30% of all smokers will develop COPD. (Lokke, Lange et al. 2006, Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2015.) According to WHO, COPD is the third leading cause of death in the world, responsible for 5.6% of all deaths globally. (World Health Statistics 2008) COPD patients also die younger than population in general. Although COPD is a chronic, progressive disease, the Global Initiative for Chronic Obstructive Lung Disease (GOLD) workforce has emphasized that COPD is preventable and treatable. Smoking cessation is a cornerstone in both prevention and treatment of COPD patients. (Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2015.)

Generally it takes years or decades of tobacco smoke exposure for COPD to develop and thus majority of COPD patients are middle-aged or older. Although COPD has been predominantly a disease of men, in the developed world the gender difference is disappearing and in some countries the mortality of women has exceeded that of men. (Chapman, Mannino et al. 2006) Other chronic diseases are more common among COPD patients than in age-matched general population. Low-grade systemic inflammation, poor oxygenation and physical inactivity might be the underlying reasons for high prevalence of comorbidities. Cardiovascular diseases and psychiatric conditions are particularly common. (Barnes, Celli 2009)

COPD diagnosis is based on exposure to tobacco smoke, typical pattern of symptoms and lung function measurements that show the level of obstruction and its reversibility. There are several classifications for disease stages; the most widely used is the 4-class GOLD classification that is based on spirometry measurements, severity of symptoms and dyspnea and the frequency of exacerbations. (Global Initiative for

Chronic Obstructive Lung Disease (GOLD) 2015.) The earlier versions of disease stage classifications were based solely on spirometry: GOLD classification on FEV1 value and FEV1/FVC-ratio and 4-class Finnish criteria classification on FEV1 values.

COPD does not develop in all smokers, which points to genetic susceptibility to COPD. It has been estimated that the overall heritability of COPD is approximately 40%. (Zhou, Cho et al. 2013) Genetic markers that would explain COPD development, airflow obstruction, smoking intensity and other aspects related to COPD are searched for with growing intensity.

The main focus of the study was on COPD. Comprehensive phenotypic data was collected from medical records and combined with a genetic polymorphism in the CHRNA5/3 locus. The study design was a combination of retrospective and prospective approaches; retrospective medical records were combined with prospective questionnaire data. The aim was to identify clinical and genetic markers that would explain progression of the chronic airway disease, patients' quality of life and mortality.

2. REVIEW OF THE LITERATURE

2.1. Chronic airway diseases

2.1.1. Chronic obstructive pulmonary disease (COPD)

2.1.1.1. Definition of COPD

COPD is a chronic, progressive and largely irreversible disease affecting airways, lung parenchyma and lung vasculature. It requires exposure to noxious gases or particles that induce chronic, neutrophilic low-grade inflammation in the lungs. COPD is characterized by persistent airflow limitation caused by obstructive bronchiolitis and destruction of lung parenchyma resulting in emphysema. (Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2015.) COPD can have a varying degree of extrapulmonary symptoms and it can promote the development of comorbidities too, which are likely resulting from activated inflammatory processes and hyperinflation that puts strain especially to cardiac functions. (Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2015.)

2.1.1.2. Risk factors of COPD

The most prominent risk factor for COPD is exposure to tobacco smoke, whether from active smoking or passive exposure to tobacco smoke. In developed world more than 90% of COPD patients have significant tobacco smoke exposure. Other risk factors are occupational exposures like organic and inorganic dusts and chemical agents, outside and inside air pollution, the former coming mainly from traffic and the latter from biomass fuel and coal burnt for cooking and warming of houses. The role of other risk factors apart from tobacco smoke is pronounced especially in the developing world. (Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2015.)

The length of exposure to tobacco smoke or other noxious particles or gases varies greatly between patients, but needs to be significant to cause COPD. Finnish national guidelines recommend to examine for alfa-1-antritrypsin deficiency, if a patient had smoked less than 20 pack years or if (s)he is younger than 45 years. (Finnish Current Care Guidelines 2009)

2.1.1.3. Prevalence of COPD

The majority of COPD patients is men, largely due to the higher number of smokers among men. A pooled worldwide evaluation based on lung function measurements in patients with clinical symptoms from year 2006 estimated that 9.8% of men and 5.6% of women have COPD. (Halbert, Natoli et al. 2006) Because the development of

COPD generally requires a long exposure of either tobacco smoke or to other noxious gases or particles, COPD is usually diagnosed in middle-aged or elderly people. The rising number of smoking women has increased the number of women diagnosed with COPD. The increase of female COPD leads to elevation of COPD exacerbations and deaths. In 2006 it was reported for the first time that more women than men die of COPD in Northern America. (Chapman, Mannino et al. 2006) In the developing world, exposure to indoor air pollution coming from open fires or stoves increases women's portion of COPD patients. Country-specific COPD prevalence estimates vary between 8–13% in Western countries. (Halbert, Natoli et al. 2006) In Finland, approximately 5–9% of population has COPD. The prevalence is higher among men. (Vasankari, Impivaara et al. 2010, Kotaniemi, Sovijarvi et al. 2005) COPD prevalence is on the rise globally, but active prevention strategies may slow down this trend. The Finnish National Programme for Chronic Bronchitis and Chronic Obstructive Pulmonary Disease took place in Finland during years 1998–2007. During this time no increase in COPD was observed. Smoking prevalence decreased 3% among study population. There was also a reduction in COPD-related hospitalizations. (Kinnula, Vasankari et al. 2011)

2.1.1.4. Diagnosis of COPD

The diagnosis of COPD is set on the basis of exposure, symptoms and lung function measurements. The most typical symptoms of COPD are cough, sputum production, and dyspnea upon exertion. GOLD has defined the diagnostic criteria that are widely used in national guidelines. (Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2015.) FEV1 (forced expiratory volume in 1 second) ratio to FVC (forced vital capacity), FEV1%, under 0.70 after bronchodilation shows non-reversible obstruction and is thus the diagnostic limit for COPD. In addition to FEV1/FVC ratio, FEV1 values that illustrate the level of obstruction are required for the diagnosis. (Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2015.) GOLD guidelines from the year 2007 and 2011 divide the level of obstruction to four classes according to FEV1 post-bronchodilation values: mild, moderate, severe and very severe obstruction with FEV1 values of predicted of >80%, 50–80%, 30–50% and <30% respectively. The new classification of disease severity is based on spirometry, severity of symptoms, severity of dyspnea, and frequency of exacerbations. Inclusion of these clinical factors to the classification of disease severity in addition to lung functions was an important change compared to the earlier guidelines. (Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2015.) Most countries use the GOLD obstruction classification in their national guidelines; the Finnish guidelines were updated in 2014. (Kankaanranta, Harju et al. 2015) Earlier Finnish guideline criteria used only FEV1 of predicted to estimate the level of obstruction. Stages of obstruction were normal, mild, moderate and severe with FEV1 of pred values of >80%, 65–80%, 40–65% and >40% respectively. (Viljanen, Halttunen et al. 1982)

The reference values for lung functions are important in diagnostics, because they are used in calculating the level of obstruction. Reference values are collected from

large population samples. In 2012, the first global reference values were published as a result of Global Lung function Initiative (GLI2012). (Quanjer, Stanojevic et al. 2012) Even though ethnic background has been taken into account, it has turned out that the new reference values do not represent all nationalities equally well. (Backman, Lindberg et al. 2015, Pereira, Duarte et al. 2014, Kainu, Timonen et al. 2015) Kainu et al found that in Finnish population, the global reference values tend to underestimate lung functions; measured FVC values were 6% and 5% higher and FEV1 values 4% and 3% higher than predicted among men and women, respectively. As a conclusion they recommended the use of new national reference values, which were published in 2015. (Kainu, Timonen et al. 2015) The old Finnish reference values collected in late 1970's were collected only from working population and the results were extrapolated to cover the elderly. (Viljanen, Halttunen et al. 1982) This led to exaggeration of the level of obstruction in the elderly as the decline in lung functions is not strictly linear. The new reference values are a great improvement because they are collected also from the elderly (≤83 years) and thus remove the problem. (Kainu, Timonen et al. 2015)

Irrespective of whether the reference values are national or global, they are still always a compromise; a representation of "average" lungs of a study population. In real life people have lungs of different sizes, e.g. lung tissue development during fetal period and in early childhood affect the size of adult lungs. Smaller lung function values can also result from healthy but small lungs. (Gibson, Simpson 2009) These individuals are, however, more easily diagnosed with airway diseases. Especially elderly people can have problems with spirometry technique, which results in inaccurate results. In a Polish study, only one third of elderly patients performed spirometry without any errors. (Czajkowska-Malinowska, Tomalak et al. 2013) Still, with new technology and well-trained staff the quality of spirometry among elderly can be as good as among younger patient groups. Overweight and constriction of chest movements are common problems among the aging population and can also lead to inaccurate measurements. (Haynes 2014)

Imaging, especially high-resolution computer tomography (HRCT) is becoming more and more important in COPD diagnostics. Although lung functions and the level of obstruction affect the severity of the disease, the prominent pathophysiological mechanism that causes the disease can differ. Similar lung functions can result from destruction of lung parenchyma (emphysematous changes and gas trapping) or from inflammation, remodelling and damage of small airways, or – most often – as a combination of these two. High-resolution computer tomography is used as a standard imaging technique to find out the pathophysiological changes in lungs (Figure 1). (Han, Kazerooni et al. 2011)

Underdiagnosis is a prominent challenge in COPD. First, smokers with "smoker's cough" or similar symptoms do not often consider these as a sign of a serious disease and second, spirometry measurements are not performed in a required scale. (Lamprecht, Soriano et al. 2015) Lindberg et al found in their study that even though patients had been to a doctor with COPD related symptoms and complaints and fulfilled the diagnostic criteria of the disease, only a minority received COPD

diagnosis. (Lindberg, Jonsson et al. 2005) People with a mild disease are especially underdiagnosed and they seldom receive the treatment they should. (Bednarek, Maciejewski et al. 2008) It is probable that the proportion of mild COPD cases in studies is also underrepresented, which is likely to change the picture we have of the disease in early stages.

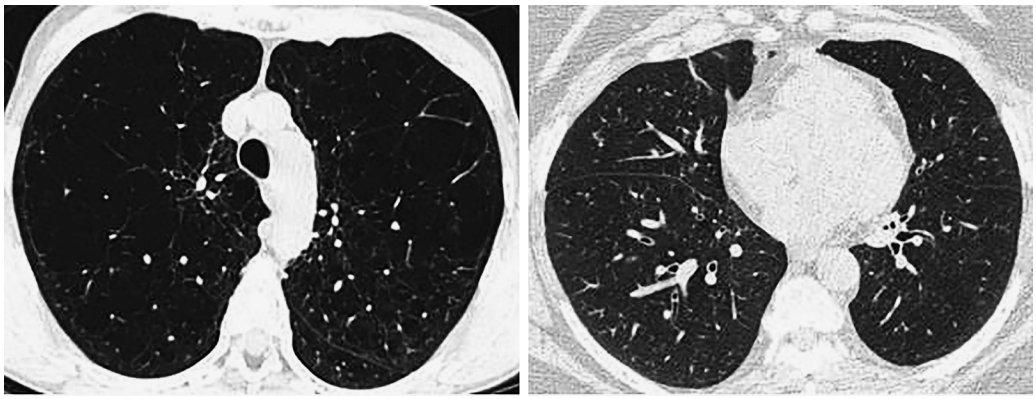

Figure 1. Two main pathophysiological mechanisms causing COPD. On the left there is clear emphysematous destruction of lung parenchyma. On the right, lung parenchyma is intact but small airways thickened. Figure from Han et al. (Han, Kazerooni et al. 2011).

2.1.1.5. Common comorbidities of COPD

COPD patients often suffer from multimorbidity. There are several reasons for the high prevalence of comorbidities. First, COPD patients' advanced age contributes to the high number of chronic conditions. Second, tobacco smoke predisposes patients to several other diseases in addition to COPD. Tobacco is a well-known risk factor for e.g. cardiovascular diseases, metabolic syndrome and diabetes. When studying low-grade inflammation the results showed that COPD patients' inflammation marker levels were increased especially in severe disease and during exacerbations. (Gan, Man et al. 2004, Wouters, Groenewegen et al. 2007, Smith, Wrobel 2014) At present it is unclear whether COPD has systemic manifestations that keep up the inflammation or if the rise of inflammation markers is an outcome of "spill overs" from inflamed lung tissue. (Barnes, Celli 2009) For example, ischemic heart disease and metabolic syndrome present also low-grade inflammation, and it is possible that the inflammation is the underlying connection between the diseases. Physical inactivity associated to dyspnea and obstruction of airways increases the risk of other chronic diseases. (Hartman, Boezen et al. 2010) COPD patients' most common comorbidities are cardiovascular diseases, lung cancer, depression and anxiety, osteoporosis, metabolic syndrome and diabetes. (Barnes, Celli 2009)

Cardiovascular diseases (CVD) are frequent among COPD patients. As mentioned above, atherosclerosis and ischemic heart disease have several common risk factors with COPD, and physical inactivity associated to airway obstruction also promotes the development of ischemic heart disease. Low FEV1 is an independent predictor of death

due to myocardial infarction regardless of smoking history, age or gender. (Barnes, Celli 2009) It has been estimated that up to 30% of stable COPD patients have some degree of heart failure. (Rutten, Cramer et al. 2005) The diagnosis of heart failure is often complicated due to symptoms shared with COPD. COPD patients' risk to develop type II diabetes is 1.5–1.8 fold compared to the general age-matched population. (Mannino, Thorn et al. 2008) The mechanisms are currently unclear but systemic low-grade inflammation possibly plays a part as well as physical inactivity. (Barnes, Celli 2009) Osteoporosis is common but underdiagnosed among COPD patients. It is common among patients who have a low BMI and low fat free mass. Osteoporosis and cachexia decrease patients' physical activity and worsen the prognosis. (Bolton, Ionescu et al. 2004) Lung cancer is very common among COPD patients because both diseases share the same main risk factor, i.e. tobacco exposure. It seems that COPD patients are very sensitive to harmful effects of tobacco smoke; they are 3–4 times more likely to get lung cancer than smokers whose lung functions have remained normal. (Sin, Anthonisen et al. 2006)

Depression and anxiety are more common among COPD patients than in general population. Estimates vary, but approximately 30% of COPD patients have clinically relevant depression or anxiety that requires medication. (Panagioti, Scott et al. 2014) Reasons behind the high numbers are still inconclusive, but social isolation due to physical impairment and worry and financial burden over poor health and several chronic conditions probably play a part. COPD patients often have a very strong nicotine addiction, and other addictions, e.g. alcohol abuse, are more common among COPD patients than in the general population. (van Eerd, van Rossem et al. 2015)This might also affect the prevalence of anxiety and depression.

Comorbidities decrease physical activity, increase the number of disability adjusted life years (DALYs) and lower patients' HRQoL. In a systematic review CVD, anxiety and depression and diabetes were all associated with poorer HRQoL. (Huber, Wacker et al. 2015) Comorbidities increase the number and duration of hospitalizations and result in higher health care costs. Comorbidities also increase COPD patients' mortality directly. (Chapman, Mannino et al. 2006, Barnes, Celli 2009)

Table 1. Prevalence of common comorbidities in COPD.

Comorbidity	Prevalence in COPD
Hyperlipidemia	46%
Hypertension	44%
Anxiety and/or depression	39%
Ischemic heart disease	29%
Heart failure	19%
Osteoporosis	17%
Diabetes	20%
Atrial fibrillation	13%
GERD	11%
Lung cancer	9%

Modified from a review article of Smith et al. (Smith, Wrobel 2014).

2.1.1.6. Smoking prevalence and cessation among COPD patients

Tobacco is responsible for the death of about 1 in 10 adults worldwide. (WHO Global burden of disease2015) Smoking is the single greatest risk factor for COPD in Western countries, including Finland. Over 90% of COPD patients have a smoking history, and 20–30% of smokers develop COPD. (Lindberg, Jonsson et al. 2005) Smoking is normally estimated with pack years that take into account both smoking time and intensity. A person that smokes one pack of cigarettes (à 20 pieces) a day for a year has smoked one pack year, as has a person who smokes half a pack a day for two years. The amount of pack years needed to develop COPD varies greatly and depends on patient's exposure to other risk factors and individual characteristics, for example genes and development and maturing of small airways and alveolar structures during fetal development and early childhood. (Postma, Bush et al. 2015)

Tobacco products have thousands of toxic compounds but the main molecule responsible for addiction is nicotine. WHO's definition of nicotine addiction is "a relapsing brain disorder characterized by loss of control over smoking and its negative impact on daily functioning". (Klinke, Jonsdottir 2014) Smoking and nicotine dependency have been shown to change neurobiology of the brain by activating nicotinic acetylcholine receptors in the brain, which leads to release of dopamine and activation of rewards system resulting in a strong addiction. (Benowitz 2010) The most common tool for evaluation of nicotine dependence is Fagerström test for nicotine dependence (FTND) (Heatherton, Kozlowski et al. 1991). The questionnaire consists of 6 questions, and the maximum score is 10. FTND score ≥6 is generally considered to show a strong addiction. (Kim, Hersh et al. 2011) In a large Spanish cohort, a high FTND score was associated with COPD diagnosis. (Jimenez-Ruiz, Miravitlles et al. 2004)

Smoking cessation and factors affecting it have been widely studied. Smoking cessation rates correlate with lung functions: the lower the lung functions, the higher percentage of patients has quit. In a systematic review it was found that among COPD patients with mild obstruction 54–77% of patients smoke. Among patients with severe obstruction, the percent of smoking patients varied between 38–51%. (Tonnesen 2013) In some studies the decline of lung functions has been faster among patients that have quit smoking. The theory behind the phenomenon is that patients who are in poorer condition quit because they cannot continue their old habits, whereas patients with better health continue to smoke. This "healthy smoker" effect is seen especially in severe COPD. Studies with longer follow-up times, however, have found out that the trend has shifted after approximately a year, and after that the lung function decline has been faster with smoking patients. (Godtfredsen, Lam et al. 2008)

Studies on the role of comorbidities in smoking cessation among COPD patients are scarce. Depression and anxiety are more common among COPD patients, and their self-efficacy is lower than other smokers'. Concomitant COPD and depression or anxiety seem to result in stronger nicotine addiction and lower quitting rate. (van Eerd, van Rossem et al. 2015)

The success rate of smoking cessation varies and depends on counselling and medication received. COPD patients fail in smoking cessation attempts more often than other smokers. They have more problems with motivation and a stronger nicotine addiction. (Jiménez-Ruiz, Andreas et al. 2015) With counselling and suitable medication, the 1-year quitting rates are 25–35%, approximately two times better than without specific intervention. (Tonnesen 2013, Stead, Perera et al. 2012) Drugs that have been shown to be effective in smoking cessation are nicotine replacement therapy (NRT), bupropion and varenicline. In a meta-analysis counselling alone has OR 1.8 and borderline significance compared to care without any kind of smoking cessation intervention. Counselling combined with bupropion is significantly more effective, OR 3.3. Counselling combined with varenicline (OR 4.0) or with NRT (OR 5.1) is even more effective. (Strassmann, Bausch et al. 2009) Also therapy groups and psychological help have been proved to have some effect in quitting smoking. (Strassmann, Bausch et al. 2009) Studies regarding COPD patients' smoking cessation attempts are scarce. It is generally presumed that similar means of quitting are as adequate in COPD patients as they are in other smokers. Whether COPD patients (and especially patients with high genetic susceptibility to nicotine addiction) would have needs for special support in smoking cessation is unclear. Smoking cessation is, however, an unquestioned cornerstone in COPD treatment. Smoking cessation slows down the lung function decline, decreases respiratory symptoms and exacerbations. (Willemse, Postma et al. 2004) Smoking cessation also improves patients' HRQoL. (Postolache, Nemes et al. 2015) When studying COPD patients' treatments and interventions, only smoking cessation and oxygen therapy had a direct effect on mortality. All other interventions including medication improve lung functions, decrease exacerbations or increase HRQoL but do not decelerate the disease progression. (Russell 2014)

2.1.1.7. Physical activity and pulmonary rehabilitation in COPD

The role of physical activity in COPD patients' well-being has been the focus of attention in recent years. The importance of physical activity as a part of disease management has been promoted and noted in international and national treatment guidelines. (Kankaanranta, Harju et al. 2015, Watz, Pitta et al. 2014) COPD patients' level of physical activity is lower than in healthy age and gender matched population. The duration, intensity and counts of daily physical activity were all lower among COPD patients. (Vorrink, Kort et al. 2011) It has been estimated that the percentage of physically active COPD patients varies between 30–60%. (Watz, Waschki et al. 2009, Spruit 2014)

Studies to find determinants for inactivity in COPD have been done, but most of them are cross-sectional and therefore show only association, not causality. Step counters and activity monitors give access to objective data on physical activity that can be validated. (Spruit, Singh et al. 2013) In a large part of the studies physical activity has been estimated from questionnaires. Questionnaires are an inexpensive and effective way for gathering data especially in large studies and when retrospective data is needed. In these studies, however, the risk of recall bias and the questionnaires' ability to capture the actual level of physical activity has to be taken into account.

The effect of lung functions on physical inactivity has been widely studied, usually with the pretension that the level of lung function decline would have a strong effect on physical activity. Surprisingly, lung function decline has explained only a small proportion of the variation in physical activity, and the strength of the associations has been weak or moderate. Also diffusion capacity has shown weak to moderate associations to physical activity. (Watz, Pitta et al. 2014) It seems that the severity of the obstruction, defined by lung function decline, is not strongly associated with physical activity levels.

Comorbidities' effect on physical activity has been studied in several but often quite small study populations. In multivariate models, concomitant heart disease and diabetes have had independent explanatory value for physical inactivity. (Watz, Waschki et al. 2008, Garcia-Aymerich, Felez et al. 2004, Andersson, Stridsman et al. 2015) Several comorbidities that have been analyzed have not had an association with physical activity, e.g. arterial hypertension or anemia. (Watz, Waschki et al. 2008) The role of fatigue, lack of motivation and depression in physical inactivity has been studied but the results are inconsistent. Some studies show association between depression and physical inactivity while others fail to do so. (Watz, Pitta et al. 2014) Among newly diagnosed COPD patients, comorbidities were more strongly associated with physical inactivity than lung functions. (Van Remoortel, Hornikx et al. 2014)

Breathlessness or dyspnea is a primary symptom when a COPD patient starts to exercise. It is also one of the main reasons limiting physical activity and leading to inactivity. Dyspnea is most often measured with Modified Medical Research Council's (MMRC) dyspnea scale. (Bestall, Paul et al. 1999) High scores on the MMRC scale are associated with physical inactivity in several studies. (Watz, Waschki et al. 2009, Waschki, Spruit et al. 2012)

COPD patients' capability to exercise is usually measured with the 6 minute walking distance test. Poor results in the test had a moderate positive association with physical inactivity but were unreliable in identifying physically inactive patients. This suggests that physical inactivity does not depend on physical form (van Gestel, Clarenbach et al. 2012) Several studies show that low HRQoL is associated with inactivity. (Waschki, Spruit et al. 2012, Waschki, Kirsten et al. 2015, Esteban, Quintana et al. 2010) A longitudinal study by Esteban et al shows that improvement of physical activity was associated with better scores in HRQoL questionnaires. (Esteban, Quintana et al. 2010)

Sociodemographic factors, like the level of education and socioeconomic status, can have an effect on physical activity on COPD patients. Interestingly, this association seems to be opposite to that of healthy counterparts: among COPD patients, higher socioeconomic status is associated with lower physical activity. (Garcia-Aymerich, Felez et al. 2004) Extreme weather conditions and a high level of pollutants are also associated with a decreased level of physical activity. (O'Shea, Taylor et al. 2007, Alahmari, Mackay et al. 2015)

Physical inactivity leads to faster lung function decline compared to physically active patients. (Pelkonen, Notkola et al. 2003, Garcia-Aymerich, Lange et al. 2007, Garcia-Aymerich, Lange et al. 2008) At the same time, physically inactive patients' lung function decline has been shown to be more rapid than that of physically active patients'. (Waschki, Kirsten et al. 2015) Physical inactivity has also been shown to have a direct effect on mortality. (Garcia-Aymerich, Lange et al. 2006) In a longitudinal study Waschki et al found physical inactivity to be the strongest predictor for all-cause mortality. (Waschki, Kirsten et al. 2011)

Several variables can be either determinants leading to physical inactivity or outcomes resulting from inactivity. Hospitalizations due to exacerbations are associated with decreased physical activity but it is also shown that physical inactivity increases the risk of exacerbations. (Pitta, Troosters et al. 2006) Severe bronchial obstruction and low FEV1 measurements have been associated with physical inactivity. Similar duality can be seen with e.g. comorbidities. Several comorbidities lower the level of physical activity and at the same time physical inactivity leads to the increase of comorbidities and also to an increase in their severity. The research of physical activity among COPD patients calls for longitudinal studies to answer these questions.

The definition of pulmonary rehabilitation is that it is a "comprehensive intervention to improve the physical and psychological condition of people with chronic respiratory disease and to promote the long-term adherence to health-enhancing behaviors". (Spruit, Singh et al. 2013) The goals of pulmonary rehabilitation thus aim to improve patients' physical shape and activity and also educate people on how to cope with the disease on a psychological level and improve their quality of life. Pulmonary rehabilitation has been repeatedly shown to increase exercise capacity and decrease sensation of dyspnea. (McCarthy, Casey et al. 2015, Nici, Donner et al. 2006) Decrease in exacerbations and improvement in HRQoL has also been reported. (Postolache, Nemes et al. 2015, Puhan, Scharplatz et al. 2009) It seems, however, that these beneficial changes have not necessarily lead to an increase in physical activity. Out of 10 studies analyzing the association between received rehabilitation and physical activity, only 4 studies found a significant association and 6 studies did not. (Watz, Pitta et al. 2014) It is clear that other matters apart from improvement in exercise capacity need also to be taken into account. It seems that patients' motivation and attitude towards physical activity plays a large part in an individual's physical activity levels and exercise habits. When studying the long-term effects of pulmonary rehabilitation, it has been found that the rehabilitation's effect on exercise capacity is lost approximately in a year, but the effect on increased quality of life and decreased sensation of dyspnea lasts longer. (Lacasse, Martin et al. 2007)

2.1.1.8. COPD exacerbations

COPD exacerbations are defined as acute events that are characterized by a worsening of respiratory symptoms and that require change in patient's medication. 60% of exacerbations are caused by viral or bacterial respiratory infections. Air pollution is also a common inducer of exacerbations. (Wedzicha, Seemungal 2007) Frequent

exacerbations are an indicator for poor prognosis and rapid decline of lung functions and they can cause permanent increase in COPD symptoms. The risk of mortality in patients having 3 or more exacerbations per year compared to patients with no exacerbations is high (HR 4.1 (CI 1.8–9.4)). (Soler-Cataluna, Martinez-Garcia et al. 2005) Exacerbations can occur even among patients with mild obstruction, but patients with high dyspnea scores, severe lung function impairment and comorbidities suffer more often from frequent exacerbations. The risk of exacerbations rises with age, and women have a higher risk of having exacerbations than men. (McGarvey, Lee et al. 2015) COPD patients with frequent exacerbations have also a significantly lower HRQoL and they are less physically active compared to patients without exacerbations. (Wedzicha, Brill et al. 2013)

2.1.1.9. Prognosis of COPD

COPD is a chronic disease that causes irreversible destruction of lung parenchyma and also thickening and fibrosis of bronchioli walls through on-going low-grade inflammation. The speed of disease progression varies greatly between individual patients. Exposure to tobacco smoke and other noxious materials maintains inflammatory processes and accelerates lung function decline. Thus smoking cessation slows the disease progress and improves patients' general well-being. In a large follow-up study it was found that smoking cessation also decreases mortality directly. (Anthonisen, Skeans et al. 2005) Other studies have found decrease in mortality after smoking cessation in mild to moderate COPD patients, but in severe COPD the mortality of quitters has been higher than in patients who still continue to smoke. (Godtfredsen, Lam et al. 2008) Because tobacco smoking is a shared risk factor for COPD and most comorbidities, e.g. cardiovascular diseases, smoking cessation decreases also their prevalence. Variable extrapulmonary effects may contribute to disease severity in individual patients. Having several comorbidities worsen the prognosis significantly. Exacerbations also worsen COPD prognosis, especially if they are frequent or require in-patient care. (Celli 2010)

Table 2. Hazard ratio for mortality in COPD patients with comorbidities.

Comorbidity	HR for mortality
COPD alone, no comorbidities	1.0
Ischemic heart disease	1.3–1.5
Heart failure	1.3–1.9
Pulmonary hypertension	1.3–1.4
Atrial fibrillation	1.6
Diabetes	1.5–1.7
Anxiety	13.8
Lung cancer	2.0
Lung fibrosis	1.5
Gastric/duodenal ulcers	1.3

Modified from Smith et al. (Smith, Wrobel 2014).

2.1.1.10. Burden of the disease and HRQoL in COPD

Disability adjusted life years (DALYs) are a numeric representation that combines years that are lost due to premature death and healthy years lost due to the disability caused by the disease. It has been estimated that 3.4% of all DALYs in Western Europe are due to COPD, and COPD has been estimated to become the fifth most common cause of chronic disability worldwide by the year 2020. (Lopez, Shibuya et al. 2006) The economic burden of COPD is significant; global estimates vary between 0.2–1.4 billion USD annually. (Chapman, Mannino et al. 2006)

Health related quality of life (HRQoL) is a patient's estimate of their current health status and well-being. Both lung specific and general HRQoL can be assessed (more on HRQoL questionnaires in chapter 2.3.). Several studies have found that COPD patients have not only lower lung specific HRQoL, but also significantly lower general HRQoL compared to healthy age-matched counterparts. In a Norwegian study COPD patients had significantly lower scores on all HRQoL components and also lower scores on physical and mental health summary components. (Bentsen, Rokne et al. 2013) Poor HRQoL is associated with poor prognosis and survival in COPD patients. (Antonelli-Incalzi, Pedone et al. 2009)

A recent meta-analysis found controversial results with regard to the effect of age. In some studies older age had a weak negative correlation with HRQoL, in some studies younger patients' had worse HRQoL. Overweight didn't correlate with poor HRQoL; underweight, on the other hand, did. (Tsiligianni, Kocks et al. 2011) It is likely that underweight patients are cachexic and have poor physical health, which reflects on their quality of life. Current smoking was found to correlate with poor HRQoL in most of the studies. (Tsiligianni, Kocks et al. 2011) Gender also plays a role in quality of life; women tend to have lower HRQoL than men. (Kanervisto, Saarelainen et al. 2010) Self-management techniques and interventions as well as good understanding of the nature of the disease and feeling in control with the management of the disease have a positive effect on HRQoL. (Zwerink, Brusse-Keizer et al. 2014, Weldam, Lammers et al. 2014)

A meta-analysis containing six studies and focusing on psychiatric conditions found that both depression and anxiety predict low HRQoL in COPD patients. (Blakemore, Dickens et al. 2014) The role of psychiatric conditions seems to be a strong factor affecting HRQoL; it shows a consistent connection to low HRQoL scores in several studies. (Tsiligianni, Kocks et al. 2011, Ekici, Bulcun et al. 2015)

The level of obstruction (e.g. GOLD classes) is only a weak indicator for quality of life and correlates with HRQoL only when the obstruction is severe. Health status evaluated with BODE index did have a stronger correlation with HRQoL. Previous exacerbations, even ones needing hospitalization, have only a weak negative correlation to HRQoL. (Tsiligianni, Kocks et al. 2011) Sensation of dyspnea, usually evaluated with the MMRC scale, has a strong correlation with poor HRQoL. Also other symptoms, like chronic cough, sputum production and wheezing, correlate with

low quality of life but correlation is not as strong as with dyspnea. (Tsiligianni, Kocks et al. 2011, Ekici, Bulcun et al. 2015)

2.1.1.11. Mortality

COPD patients die younger than their healthy age and gender matched counterparts. (Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2015.) According to WHO, COPD was the third leading cause of death in the world in 2012, being responsible for 5.6% of all deaths. (WHO Global burden of disease2015) In Finland, 1150 persons died of COPD in 2013 (death rate 21.7/100,000), 88% were 65 years or older. (Statistics Finland: Causes of death.2014) Lung function decline in COPD patients is prognostic for death; the poorer the lung functions, the higher the risk of dying. (Jenkins, Jones et al. 2009) After diagnosis the 10-year survival rate is approximately 50%, and more than one-third of patients die due to respiratory insufficiency. (Anto, Vermeire et al. 2001) In TORCH, a large longitudinal multicentre clinical trial, patients' 3-year mortality risk was 11% in moderate obstruction, 15% in severe and 24% in very severe obstruction. (Jenkins, Jones et al. 2009) In a large Finnish study even mild obstruction increased the risk of death (HR 1.3). In very severe obstruction HR was 2.9. (Mattila, Vasankari et al. 2015) Although there is a clear association between worsened lung functions and mortality, COPD patients are reported to die because of non-pulmonary reasons, not of COPD itself. In their study Fabbri et al found the most common causes of death to be cardiovascular diseases (approximately 25% of COPD patients' deaths) and lung cancer (approximately 30% of deaths). (Fabbri, Luppi et al. 2008) In TORCH, cardiovascular diseases were the cause of death in 27% of all deaths, respiratory causes 35% (of which COPD 27% and pneumonia 8%) and cancer 21% (lung cancer 14%, other cancers 7%). (McGarvey, John et al. 2007) Smoking cessation decreases COPD patients' mortality rates but ex-smokers' mortality risk does not reach the risk level of people who had never smoked. (Godtfredsen, Lam et al. 2008)

The reliability of statistics on COPD deaths should be revised critically: as COPD is underdiagnosed during patients' lives, is it underdiagnosed as a cause of death, too. Even though COPD might be the main reason of death, other diagnoses like pneumonia are often used. Also common comorbidities, such as left ventricular heart failure, cardiac arrhythmias or ischemic heart disease, are erroneously but commonly used instead of COPD. This leads to the underestimation of COPD's importance and the magnitude of impact the disease has. (Lopez, Shibuya et al. 2006)

2.1.1.12. COPD genetics

COPD is a multifactorial disease, and genes play a part in its development. Family-based and twin studies have shown that lung function is partly heritable. (Hukkinen, Kaprio et al. 2011) Not all smokers develop COPD, so apart from environmental exposure genetic susceptibility is needed too. The heritability of phenotypes related to COPD (lung functions, emphysema) has been estimated and the overall heritability of

COPD disease status was approximately 40%. (Zhou, Cho et al. 2013) Usually exposure to tobacco smoke or other irritants is nevertheless compulsory for the disease to develop. An exception to this "rule" is the best-known genetic syndrome causing COPD; α-1-antitrypsin deficiency. Enzyme α-1-antitrypsin protects the lung parenchyma against proteolytic damage from neutrophil elastase. There are several mutations causing the deficiency, but the most frequent is located in SERPINA1 gene in chromosome 14q31. The mutation causes polymerization of molecules and promotes their storage in liver cells, which leads to too low serum concentration resulting in accelerated destruction of lung parenchyma. (Stoller, Aboussouan 2005) In Europe the prevalence of α-1-antitrypsin deficiency has been estimated to be approximately 1/2500 people. (Fregonese, Stolk 2008) In Finland the prevalence of mutated alleles is similar to neighbouring countries like Sweden and Estonia. (Häggblom, Kettunen et al. 2015)

Apart from α-1-antitrypsin deficiency, other genetic associations to COPD are rather weak and their mechanisms are unclear. Zhou et al. has estimated that 38% of COPD development is genetic, but only a few genes have been identified and their functions are known. (Zhou, Cho et al. 2013) The effect of epigenetic and post-translational mechanisms in the development of COPD is unclear for the time being.

Meta-analyses on candidate gene studies have found reliable associations between COPD and a few genes, most of which either regulate inflammatory responses (e.g. tumor necrosis factor α, TNFA and transforming growth factor β1, TGFB1), are involved in protease-antiprotease activity (e.g. matrix metalloproteases, MMPs) or are antioxidant genes (e.g. glutathione transferase subunits, GSTP1, GSTM1). (Smolonska, Wijmenga et al. 2010) It seems that ethnicity also plays a part in the disease process, e.g. TNFA minor allele associates with an increased risk of COPD in Asian populations. (Smolonska, Wijmenga et al. 2010)

There is a large number of genetic studies with usually quite small study populations and weak associations, but three main susceptibility loci containing 4 genes have been repeatedly shown to associate with COPD related characteristics (pack years, emphysema or lung functions) in genome-wide association studies (GWAS). (Pillai, Kong et al. 2010b) The loci were found through GWAS method and thus didn't have a previous hypothesis on the genes' effects on COPD. Loci contain hedgehog interacting protein gene, HHIP, in chromosome 4 and another locus in chromosome 4 with FAM13A gene, the function of which is presently unknown. CHRNA3/5 gene coding for nicotinic receptor and IREB2 coding for iron responsive element are located in chromosome 15 and are in tight linkage disequilibrium, and are thus considered in literature as one locus. (Pillai, Kong et al. 2010b) CHRNA3/5 is associated with smoking behavior; cigarettes smoked per day and pack years. It's also significantly associated with lung functions and emphysema (quantified by CT scan) among COPD patients and smokers without COPD diagnosis. (Pillai, Kong et al. 2010b, Kaur-Knudsen, Bojesen et al. 2014) Minor homozygosity was associated with both low FEV1 and FEV1/FVC ratio and also clinically significant emphysema. HHIP

locus associated with FEV1/FVC ratio and also with emphysema. (Pillai, Kong et al. 2010a)

To validate to findings of smaller studies, large data sets are needed. One of the most comprehensive and interesting genetic COPD studies at the moment is COPDGene study, a large American longitudinal study with more than 10,000 cases and controls. It started in the year 2008 and is still ongoing. In addition to genetic data, also phenotypic characteristics are gathered from the study subjects. (Regan, Hokanson et al. 2009) Over a hundred articles have already been published on different aspects of COPD as a part of COPDGene consortium. (COPDgene Consortium2015) COPDGene study is an example of longitudinal, prospective study design that combines phenotypic and genetic data and has good statistical power due to a large number of participants. The cohort consists of non-Hispanic white and African American population, so it remains unclear whether the results could be generalized to e.g. Hispanic or Asian populations. (Regan, Hokanson et al. 2010)

2.2. Asthma

2.2.1. Definition and diagnosis of asthma

Asthma is a widespread, multifactorial chronic disease of the airways. It is characterized by chronic inflammation and reversible obstruction of bronchi and bronchioli. It has complex and varying mechanisms of pathogenesis and several phenotypes that can differ on the onset, clinical manifestations and treatment response. Asthma symptoms include wheezing, coughing and dyspnea that vary in intensity over time. The clinical definition of asthma is based on lung function values and clinical symptoms. Diagnostic lung function measurements are increased diurnal variation of airflow (PEF over 20%) and obstruction of airways that is relieved by inhaled short acting β2-agonists; the effect of bronchodilation needs to be ≥ 12% in FEV1 value or ≥15% in PEF measurement. The hyperreactivity of bronchi can be tested with histamine or methacholine provocation test, in which 15% decrease of FEV1 is diagnostic. The most common symptoms of asthma are wheezing, dyspnea, chest tightness and coughing, predominantly at night or early morning. (Finnish Current Care Guidelines in Asthma2013)

2.2.2. Adult-onset asthma

According to WHO there are 235 million people suffering from asthma worldwide. It has been estimated that the prevalence of asthma in older adults (≥65 years) is between 6% and 10% in the developed world. (Gibson, McDonald et al. 2010) In Finland the prevalence of asthma in the elderly is approximately 10%. (Kainu, Pallasaho et al. 2013) It has been traditionally thought that the onset of asthma typically takes place in

childhood or adolescence but asthma can be diagnosed at any age. In Finland the group that receives most new asthma diagnoses are 60-64 years old women. (Finnish Current Care Guidelines in Asthma2013) Asthma presenting itself in adulthood is defined as "late onset asthma" and unlike asthma in childhood it is not usually associated with allergies. (Global Initiative for Asthma, GINA 2015) Asthma symptoms can change, reduce or evolve in time. Patients who have had asthma since childhood or youth do not always have symptoms throughout their life, although it is possible. Older asthmatics are a heterogeneous population with large variation in disease onset and symptomatology, and their disease is affected by their life histories, e.g. occupation and smoking. (Gibson, McDonald et al. 2010) There are several asthma phenotypes that are common in adult-onset disease. Adult-onset asthma can be eosinophilic and such disease is often severe in adults. Adult-onset asthma can be also exercise-induced, obesity-related or neutrophilic. (Wenzel 2012) One interesting subtype of adult-onset asthma is asthma with persistent airflow obstruction. Typical features of this subtype are poor response to medication and low post-bronchodilation lung function values. There is an ongoing debate whether this patient group is actually ACOS patients or a subgroup of asthma. (Global Initiative for Asthma, GINA 2015, Konstantellou, Papaioannou et al. 2015) Unlike in asthma that starts in childhood, there is very little knowledge on the prognosis of adult-onset asthma. A recent review concluded that it seems that remission is hard to achieve in adult-onset disease but to draw definite conclusions on prognosis of adult-onset asthma, more longitudinal studies on the subject are needed. (Tuomisto, Ilmarinen et al. 2015)

2.2.3. Mortality

In the year 2011 the death rate related to asthma was 5/100,000 in the whole world, most deaths occurring in low or lower middle income countries. In Finland asthma mortality has decreased steadily; in the year 2013 82 persons died of asthma in Finland (mortality rate 1.55/100,000). 74 (90%) of the patients were 65 years or older and one of them was <40 years old. (, Statistics Finland: Causes of death.2014) WHO estimates that although the mortality in children and young to middle-aged adults will keep decreasing, the mortality in the elderly will increase in the future. (WHO Global burden of disease 2015) The mortality in the over 70-year-old patient group was estimated to be 13/100,000 in the European region in 2015. (WHO Global burden of disease 2015)

2.3. Asthma and COPD overlap syndrome (ACOS)

The simultaneous prevalence of asthma and COPD (asthma and COPD overlap syndrome, ACOS) is a new, rising consept in pulmonary medicine. First review articles on the subject have been published in 2005 (Guerra 2005). The interest for the overlap

syndrome has risen from the clinical notion that asthma or COPD diagnoses do not cover all patients with chronic obstructive airway disease.

2.3.1. Pathogenesis of ACOS

The Dutch hypothesis, formulated in the 1960s, has been offered as an explanation for ACOS. It states that the various forms of airway obstruction are different expressions of a single disease entity that leads to asthma, COPD, or an intermittent disease depending on the different environmental factors that activate shared genetic factors. (Postma, Boezen 2004, Sluiter, Koeter et al. 1991) Shared genetic markers between asthma and COPD have been searched for. A meta-analysis that combined several GWAS was done in 2014 but common genetic markers for asthma and COPD were not found. (Smolonska, Koppelman et al. 2014) Other explanations to the development of ACOS are common risk factors for asthma and COPD, e.g. smoking accelerating lung function decline and respiratory infections in childhood that can lead to incomplete lung growth. (Gibson, Simpson 2009) There are also critiques to the whole concept of overlapping syndrome, questioning whether ACOS is simply COPD with frequent exacerbations. (Al-Kassimi, Alhamad 2013, Barnes 2006)

2.3.2. Definition of ACOS

A joint project of GINA and GOLD defines ACOS as persistent airflow limitation with several features usually associated with asthma and several features usually associated with COPD. (GINA-GOLD. Diagnosis of diseases of chronic airflow limitation: asthma, COPD and ACOS.) Patient's symptoms and clinical findings lay in between those of asthma and COPD: airflow limitation is persistent but can have larger variability than in COPD. Unlike in asthma, lung function decline is constant but can be slower than in COPD. Age at onset of ACOS is usually over 40 years. Exacerbations can be more frequent than either in asthma or COPD. Bronchial hyper-responsiveness is common resembling asthma. Inflammation can be either neutrophilic or eosinophilic. A history of allergies and atopy is common. (GINA-GOLD. Diagnosis of diseases of chronic airflow limitation: asthma, COPD and ACOS, Gibson, Simpson 2009) Table 3 summarizes characteristics of the three diseases.

ACOS can develop from severe asthma when a partially non-reversible bronchoconstriction develops and lung function decline accelerates during disease progression. Often asthmatics developing ACOS are older and have smoking history. COPD patients can be diagnosed with ACOS when their airflow obstruction is at least partially reversible.

Table 3. Differences and similarities between asthma, COPD and ACOS.

Feature	Asthma	COPD	ACOS
Age of onset	Usually childhood	Usually >40 years	Usually >40 years, symptomatology possible in earlier life
Typical symptoms	Variable symptoms, external triggers common (allergens, exercise, emotions)	Chronic and continuous symptoms, especially during exercise	Chronic and continuous symptoms with greater variability than in COPD
Lung functions	Variable airflow limitation, reversible by bronchodilation	FEV1 response to bronchodilation possible, post-bronchodilation FEV1/FVC ratio always <0.7	Not fully reversible airflow limitation but often greater variability than in COPD
Lung functions between symptoms	Normal when asymptomatic	Persistent airflow limitation	Persistent airflow limitation
Patient/family history	Allergies and asthma in childhood or in family are common	History of tobacco exposure	Often both asthma and COPD like history
Time course	Improvement often spontaneously or with treatment, may lead to fixed airflow limitation	Slow progression over the years regardless of treatment	Symptoms are partly reduced by treatment. Progression is usual.
Chest X-ray	Normal	Emphysematic changes and hyperinflation, possible thickening of bronchiole	Similar to COPD
Exacerbations	Exacerbations occur, risk can be reduced considerably by treatment	Exacerbations can be reduced by treatment. Comorbidities contribute to impairment during exacerbation.	Exacerbations may be more common than in COPD but can be reduced by treatment. Comorbidities can contribute to impairment.
Airway inflammation	Eosinophils and/or neutrophils	Neutrophils and eosinophils in sputum, lymphocytes in airways. Systemic inflammation.	Eosinophils and/or neutrophils in sputum

2.3.3. Prevalence of ACOS

There are several estimates of the prevalence of ACOS. Studies from different populations have estimated that 9–33% of COPD patients have ACOS. Prevalence of ACOS among older asthmatics varies between study populations from 6% to 35%. The numbers depend on the study populations, the diagnostic methods and ways of gathering the information. (Barrecheguren, Esquinas et al. 2015) Clinical population studies suggest the prevalence of approximately 20%. (Gibson, McDonald 2015) In

Finland, the percent of ACOS patients in large population study was 16% of diagnosed asthma and COPD patients. (Andersen, Lampela et al. 2013) In another Finnish study that focused on primary care asthma patients with smoking history, the prevalence of ACOS patients was 27%. (Kiljander, Helin et al. 2015)

2.3.4. Burden of the disease in ACOS

Attention is increasingly paid to ACOS patients because they have more symptoms (especially dyspnea and wheezing) and more frequent exacerbations than either asthma or COPD patients. Comparison to COPD patients is especially interesting because COPD patients are generally considered to be a challenging group of patients with large amount of comorbidities, exacerbations and high demand of health care services. Still, a study found that ACOS patients use up to five times more health care resources than COPD or asthma patients (Shaya, Dongyi et al. 2008). A Finnish population study found that although ACOS patients made up only 1/6 of all patients with obstructive airway diseases, they used 1/3 of the inpatient resources (Andersen, Lampela et al. 2013). The burden of comorbidities among ACOS patients is higher than among asthmatics. Whether the burden exceeds that of COPD patients is unclear. In their review Gibson et al summarized present knowledge on the topic. Of the six studies found, four showed a statistically significant increase between comorbidities among ACOS and COPD patients. (Gibson, McDonald 2015) HRQoL of ACOS patients has been shown to be poorer than that of asthmatics or COPD patients. (Miravitlles, Soriano et al. 2013) In an adjusted survival model of an American study, the risk for mortality was significantly higher among ACOS patients compared to asthma or COPD patients. (Diaz-Guzman, Khosravi et al. 2011)

2.4. Validation of genetic loci in multifactorial disease

COPD and asthma are multifactorial diseases and genes do have an effect on disease onset and severity. The genetic component of COPD development is estimated to be approximately 40% but not all genes contributing to the development have been identified. (Zhou, Cho et al. 2013) Even less is known about the mechanisms through which they cause the disease. Genes that affect different aspects related to the development of COPD, like lung functions or smoking habits, have been mostly found with GWAS. GWAS is a method for mapping genes, with the aim of finding regions and genes of interest that could contribute to the development of a disease. (Manolio 2010) GWAS have gained popularity when studying multifactorial diseases because, unlike candidate gene studies, they don't require strong a priori expectations of genes that will be tested. As the name implies, with GWAS one can cover the whole genome and find unforeseen genomic regions of interest relatively fast and with low cost. GWAS use single nucleotide polymorphisms (SNPs) as markers. SNP is a one-nucleotide difference in genetic code and consequently the smallest possible genetic

marker there is. If SNP's allele frequencies differ between cases (e.g. COPD patients) and controls, it can be assumed that the region around the SNP differs also. Thus SNPs with known loci are "signposts" that draw attention to genes in their vicinity. There are two possibilities when a difference in SNP's allele frequencies is found. First, the inspected SNP can directly cause the disease, as is case with some monogenic disease like sickle cell anemia, or the SNP is located in a gene that contributes to a development of a polygenic disease. Secondly, and more often, the studied SNP is simply located in the same locus with a gene that has an impact on the disease. (Manolio 2010) Due to laws of crossing over, loci near each other in the same arm of a chromosome tend to be inherited together, i.e. they are in linkage disequilibrium. Knowing the level of linkage disequilibrium helps in both selection of SNPs for GWAS and in determination of the region and genes one must focus on when finding a difference in SNP's allele frequencies. (Slatkin 2008)

SNPs' allele differences between cases and controls can lead to finding new genes that affect some aspect of a disease. The aim is that once the genes and their mechanisms of action have been figured out, the information can be used to develop diagnosis, treatment and prevention of a disease. This goal has, however, proven to be very ambitious. (Visscher, Brown et al. 2012) A difference in allele frequencies can in some cases be used to estimate the risk of disease. If minor allele carriers are found to have higher chance to develop a disease or condition, genetic testing can help to find patients that need to be especially focused on.

2.5. Health related quality of life (HRQoL)

2.5.1. Definition of HRQoL

Health related quality of life (HRQoL) covers a person's quality of life that is directly related to one's health or disease status. HRQoL is often assessed with self-administered questionnaires that usually cover physical, social and psychological spectrums of a person's life. HRQoL is a subjective measurement that focuses on a person's experience of a disease and the impact it has on satisfaction and quality of daily life. (Bakas, McLennon et al. 2012)

There are both general and disease-specific HRQoL questionnaires. The questionnaires aim to give objective, validated data on HRQoL. As the name implies, the goal of general questionnaires is to get a comprehensive view of the most important aspects of person's daily life and their relevance for quality of life. Disease-specific questionnaires focus on a certain disease or condition and try to measure its effects on a person's daily life.

2.5.2. General HRQoL

In Finland the most frequently used general HRQoL questionnaire is 15D-questionnaire that covers 15 dimensions of everyday life: mobility, vision, hearing, breathing, sleeping, eating, speech, elimination, usual activities, mental function, discomfort and symptoms, depression, distress, vitality, and sexual activity. (Sintonen 2001) A summary score ranging from 0 (poorest possible health) to 1 (perfect health with no complaints) can be calculated but individual scores can also be used in evaluation of a person's HRQoL. The minimum important change for 15D score is 0.015. (Alanne, Roine et al. 2015) 15D questionnaire has been compared with established HRQoL questionnaires like EQ-5D and SF-6D and the validity and reliability have been similar. (Ryynanen, Soini et al. 2013)

2.5.3. Respiratory specific HRQoL

There are several lung specific HRQoL questionnaires that focus only on pulmonary symptoms and their impact on everyday life. One of the oldest and most used is St George Respiratory Questionnaire (SGRQ), covering 76 aspects of lung associated health and symptoms. (Jones, Quirk et al. 1992) It is, however, several pages long and includes tens of items that patients need to fill out. Airway Questionnaire 20 (AQ20) is a shortened version of respiratory specific questionnaire developed for COPD patients with 20 dichotomous questions covering the most common symptoms and lack of activities among COPD patients. (Hajiro, Nishimura et al. 1999) It is gaining popularity due to short and simple questions and short answering and scoring times.

2.5.4. Strengths and weaknesses of general and specific HRQoL

General HRQoL questionnaires give a wider view of patient's well-being and coping but when studying the effects of a certain disease, they might not capture them very well, especially if the disease affects only one or a few topics of the general questionnaire and thus affects the summary score only little. Another problem is that if the patient suffers from several diseases, it is impossible to say which part of the score comes from the disease of interest. Simultaneous use of both general and disease-specific HRQoL questionnaire removes these problems and gives a good overview of a patient's quality of life and factors affecting it. (Huber, Wacker et al. 2015a)

2.6. Study designs in epidemiological research

2.6.1. Retrospective studies

Retrospective study design is used for analyzing and studying data that has existed before the study. In a retrospective study, data from a population with existing outcome is collected and analyzed in order to find out what could be the causative agents. Unlike prospective studies, in which data is collected and outcomes detected during a follow-up time, retrospective data gives access to large amounts of data from large study populations fast and with relatively low cost. (Uhari, Nieminen 2012)

Limitations of retrospective studies arise from the nature of the data. Retrospective data can be collected for other purposes than research, it can be e.g. patient data from electronic medical records or study subjects can fill in questionnaires as they recall the data. Not all data is found from all the patients, which can lead to research bias. Researchers have to make careful decisions on how to build up data sets and decide which events are the key ones that every patient needs to have. Another problem with retrospective analysis is time. There are not many (if any) common time points, that is, events that would have happened in a certain time window to all the patients. Even when a key event is defined so that only patients with a certain event at a certain time are included, the events and measurements following that are random. For example, if all patients who have received a COPD diagnosis at a certain time are included in a study, the time of following physical examination or spirometry measurement varies greatly. Not only are the measurements from different time points, but also the number of measurements between patients differ. Matters like these are completely out of researchers' hands when working with retrospective data. The only possibility is to tighten the inclusion criteria to a point where the study subjects are homogenous enough. This is always done with reduction of eligible study subjects, and if the number of suitable study candidates is small to start with, the decision is a difficult one. The strengths of retrospective data are that it is a fast and inexpensive study design. Data used in the analyses is not collected for study purposes but is accumulated over the years while treating patients. This decreases the chance of bias because patients are not selected to the study and there is no recall bias when medical records are used. On the other hand, the risk of recall bias is great when using data that study subjects have provided from memory. (Mann 2003)

One has to also keep in mind restrictions in collecting data from e.g. public health care systems and guard patients' anonymity. More of matters of privacy and study ethics in chapter 4.6., Ethical issues.

2.6.2. Prospective studies

Prospective studies focus on detecting differences or outcomes in a study population during a certain study period. The follow-up period varies depending on the outcome

of interest and can be a fixed time, e.g. 10 years, or the follow-up can continue until enough outcomes have been reported. A detailed and careful recruitment of study subjects and collection of data are important in the beginning of the study so correct causal conclusions can be made. Prospective studies have fewer sources of bias than retrospective studies. Loss of study subjects during follow-up has to be avoided by keeping the drop-out rate as low as possible. A negative side of prospective studies is that they are very time-consuming and expensive to perform compared to retrospective or cross-sectional studies. (Uhari, Nieminen 2012, Mann 2003)

2.6.3. Longitudinal versus cross-sectional studies

Both retrospective and prospective studies can be longitudinal. In longitudinal studies data is collected for a certain time period, and time can be included as a parameter in the analysis. This enables to study causalities between studied events, e.g. if a majority of patients have smoked before receiving a COPD diagnosis, it is probable that smoking is at least one of the causative agents of COPD. If data is gathered at a single time point, a study is cross-sectional. With a cross-sectional study it is possible to say that e.g. prevalence of smoking and COPD diagnosis are associated, but it is not possible to make conclusions for causality. Even though longitudinal studies are very useful and provide important data, they have problems, too. Long follow-up periods are one challenge, and the longer the follow-up time, the larger number of study subjects drop out of the study. This reduces the number of patients in the final analyses and diminishes the statistical power. It can also lead to bias in the data, if the drop-outs differ from the patients who continue in the study. (Uhari, Nieminen 2012)

3. AIMS OF THE STUDY

The main focus of this thesis is in chronic obstructive pulmonary disease, its development and prognosis.

The aim of this thesis was to characterize a real life Finnish chronic airways disease cohort. The special interest was on patients with irreversible obstruction, and in three substudies COPD and ACOS patients were analyzed together. We wanted to find out how combining of retrospective medical records data with genetic data and prospective questionnaire data would work and what would be the benefits and challenges of this kind of complex study design.

The specific aims were

- To find out the prevalence of patients with asthma and COPD overlap syndrome (ACOS) and analyze how this patient group differs from patients with only asthma or COPD diagnosis.
- To find out the level of COPD patients' physical activity, spontaneous exercise habits and received pulmonary rehabilitation and analyze their association to disease severity and progression.
- To estimate COPD patients' probability of smoking cessation and clinical characteristics explaining it and to analyze associations between smoking cessation, comorbidities and mortality.
- To study whether genetic markers associate to COPD in Finnish population. To analyze the effect of nicotinic acetylcholine receptor CHRNA3/5 polymorphism on smoking intensity, cancer and mortality among patients with long history of smoking.

4. MATERIALS AND METHODS

4.1. Chronic Airway Disease (CAD) cohort

4.1.1. Background on CAD study

CAD study was originally designed and started by GeneOS Ltd in close collaboration with Helsinki and Turku Universities. The original focus was on asthma patients; COPD patients were included into the study plan later. The institutions responsible of the CAD study were changed from GeneOS Ltd to Universities of Helsinki and Turku on year 2007 and all information on recruited patients and collected health care documents gathered by GeneOS were transferred to the two universities. Most of the research have been done in Research Unit of Pulmonary Diseases in Helsinki University.

4.1.2. Recruitment process

The recruitment took place in two regions and four hospitals in Finland. All hospitals provided special health care for respiratory illnesses. The recruitment, starting in 2005 in the Hospital District of Helsinki and Uusimaa (HUS), covering three hospitals providing specialist health care: Helsinki University Central Hospital (HUCH), Skin and Allergy Hospital and Jorvi Central Hospital. In 2007 the recruitment was performed also in the Hospital District of Southwest Finland in Turku University Central Hospital (TUCH). The recruitment ended in both districts in 2007. All asthma patients and the majority of the patients in general were recruited from the Helsinki and Uusimaa district. 39.4% of COPD patients were recruited from the Hospital District of Southwest Finland in 2007.

All patients who had received care for asthma or COPD in the abovementioned hospitals during years 1995–2006 and were aged 18-75 years old during the recruitment were approached with a letter and asked to join the study. The patients were identified from hospital databases with ICD-10 codes J45.0, J45.1, J45.8 and J45.9 (for asthma) and J44.0, J44.1, J44.8 and J44.9 (for COPD).

The patients were recruited by a two-phase mailing campaign. First, all patients who met the criteria were sent a letter asking to join the study. One reminder was sent if contact was not reached after the first letter. Patients who responded and showed interest in joining the study were then sent a detailed letter describing the study plan, aims and schedule. All patients were invited to the recruitment visit in the research center in either Helsinki or Turku University Central Hospitals. The recruitment process and number of patients are illustrated in figures 2 and 3 for Helsinki and Turku regions, respectively.

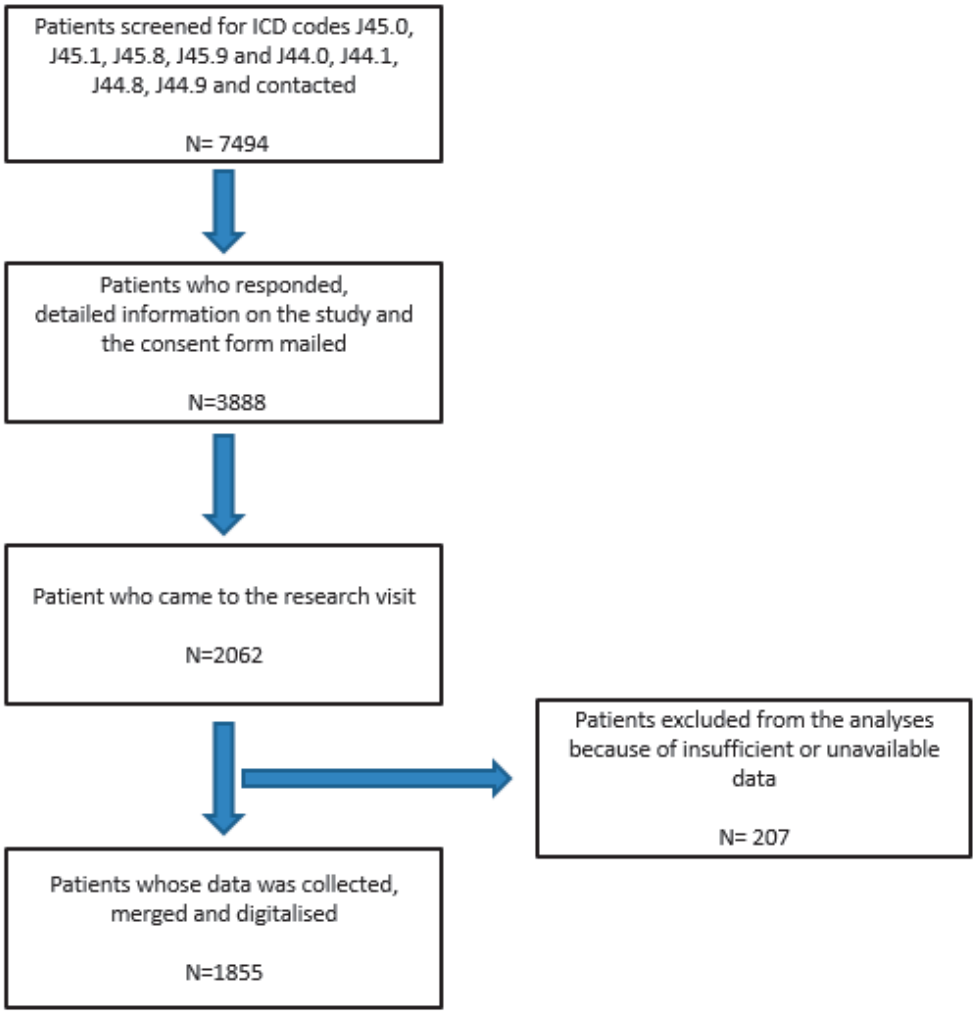

Figure 2. Recruitment process in the Hospital District of Helsinki and Uusimaa.

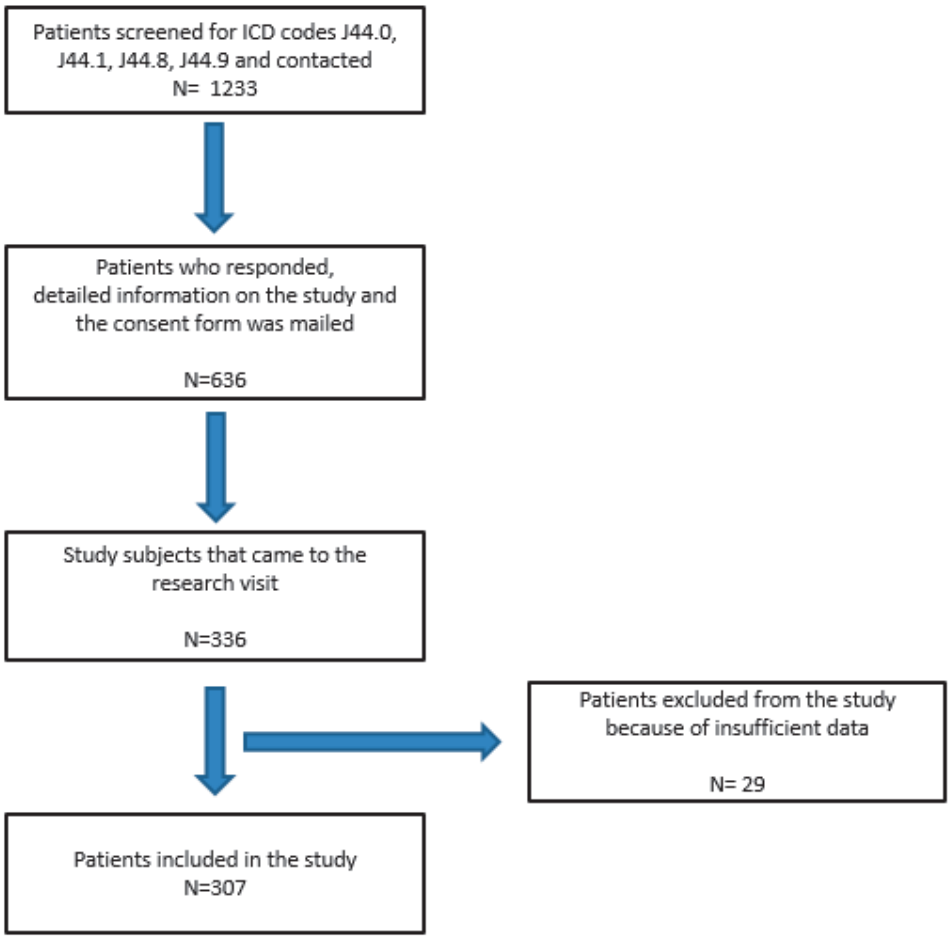

Figure 3. Recruitment process in the Hospital District of Southwest Finland.

4.1.3. Research visit

When joining the study, the participants came to a research visit and signed the informed consent. The outline of the study and aims of the research were explained to the patients by a trained research nurse. Participants gave information about their medical history from different health care providers from prior five years before the study and from prior ten years from university hospitals. Patients also filled a questionnaire regarding their general and lung specific health related quality of life (HRQoL), their work status and use of medication. During the research visit patients donated a blood sample from which DNA was later extracted. Patients also agreed to that they could be informed about and asked to join other studies later on and to answer the follow-up questionnaires every second year.

4.1.4. Collection of patients' medical records

When patients had given information about their health care providers, the research nurses contacted them and requested for patients' health care records. Once these were received, research nurses de-identified the data by removing all names, social security numbers and other identifying data from the records. After that the records were scanned and digitalized and given to the research group. When the research group started working with the data, the data of 1855 patients from the Helsinki and Uusimaa district was ready for analyzing. With rest of the patients (N=207) the medical records weren't sent or the material wasn't digitalized. In the Turku region medical records were received from all patients (N=336).

4.1.5. Collection of data from medical records

A large part of the data that was used in the analyses was collected from patients' medical records. Patients' weight and height were collected and BMI was calculated. Patients' height and gender were used in calculating reference values for lung functions using Finnish reference value equation. Smoking years and amount of cigarettes per day were collected and pack years calculated (http://smokingpackyears.com/). Spirometry measurements (FEV1, FVC and PEF) were collected and FEV1/FVC ratio was calculated with both pre and post bronchodilation values. Pre bronchodilation values were used in classifications to minimize the number of exclusions due to missing values. COPD patients were divided to GOLD-classes and to four classes according to the Finnish criteria for COPD. (Rabe, Hurd et al. 2007, Viljanen, Halttunen et al. 1982) Lung diffusion capacity values were collected. Information of other chronic diseases (cardiovascular diseases (coronary disease, cerebrovascular disease, and peripheral vascular disease), diabetes, hypertension, cancers, psychiatric conditions and alcoholism) was also gathered.

4.1.6. Follow-up of the patients

The patients are followed 10 years onwards from the beginning of the study with follow-up questionnaires. The aims of the questionnaires have been to receive more information about the patients and furthermore to evaluate the change in the answers to the questions that are repeated in every questionnaire. A more detailed explanation of the questions used in the follow-up questionnaires can be found in chapter 4.3. Study questionnaires. The questionnaires were/will be sent on follow-up years 1, 2, 4, 6, 8 and 10. Before sending the follow-up questionnaires the research nurses go through the death register and remove dead patients from the mailing lists. If response to questionnaire is not received, one reminder is sent to the patients. Apart from the follow-up questionnaires that were/will be sent to the whole cohort, a spin-off study was done with a subcohort of the CAD cohort (COPEX study, see chapter 4.1.8. Final data sets used in articles). Figure 4 illustrates the outline of the CAD study and shows the years when follow-up questionnaires were/will be sent and when COPEX study took place. The response rate to follow-up questionnaires has varied between 87% and 95% (figure 5).

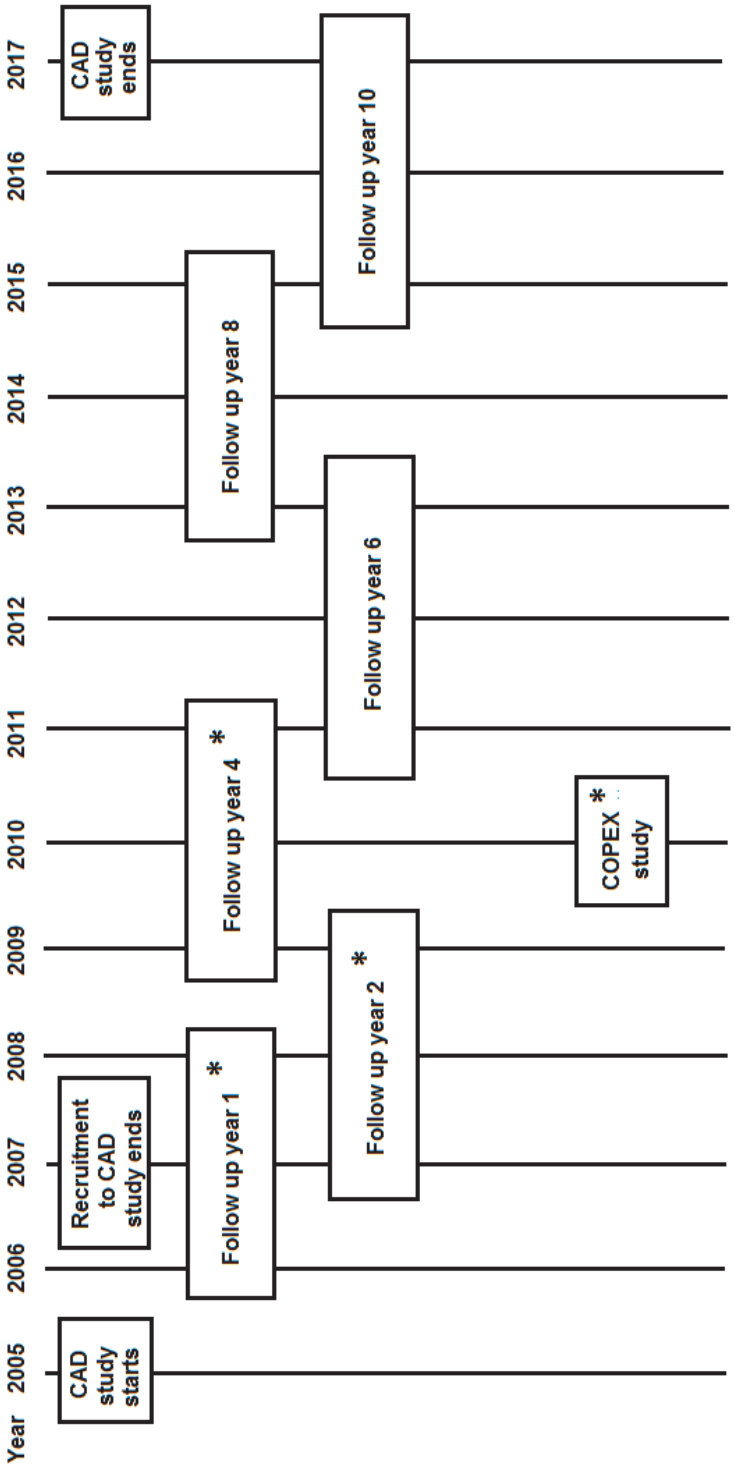

Figure 4. CAD study outline. Data used in this thesis is marked with*.

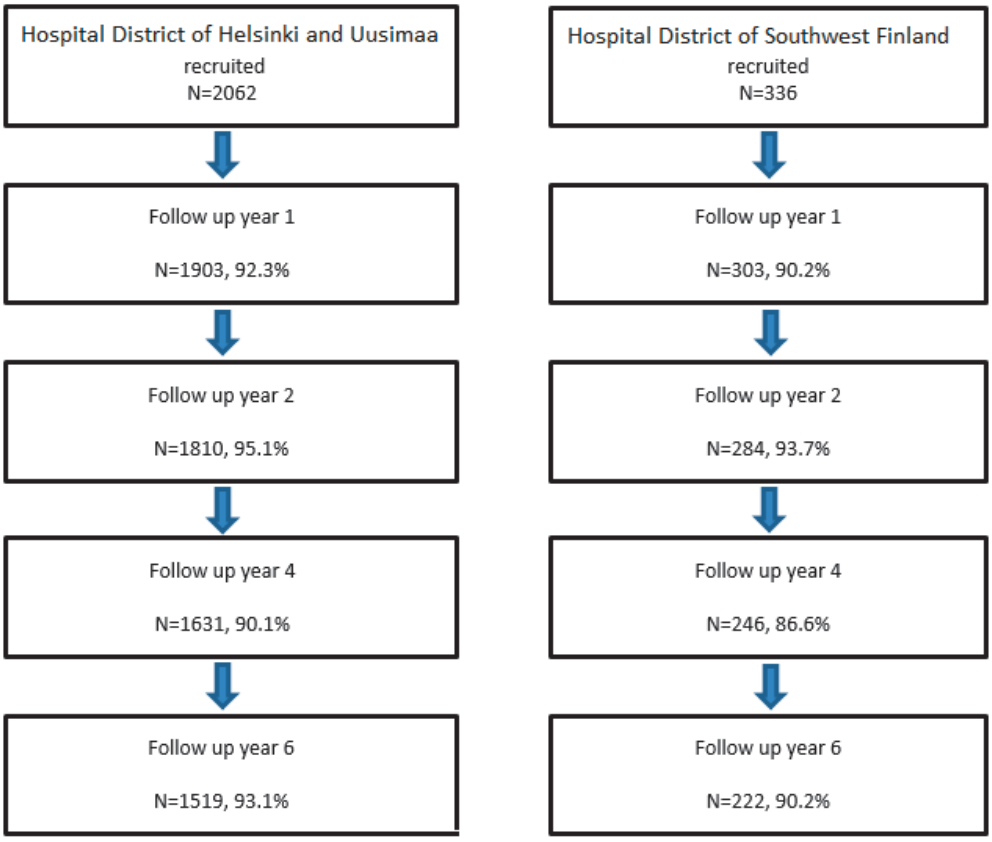

Figure 5. Follow-up response rate in Hospital Districts of Helsinki and Uusimaa and Southwest Finland (N, %).

4.1.7. Re-evaluation of diagnoses

Once the data collection from the medical records began it became clear that initial diagnoses weren't always accurate. A large proportion of asthmatics had also a COPD diagnosis, and some of the COPD patients turned out to have a primary diagnosis of asthma. 110 patients with neither asthma nor COPD diagnosis, but e.g. bronchitis or bronchopulmonary anomaly were excluded from the analyses. All medical records were read and the diagnoses re-evaluated and divided into three classes: asthma, COPD and asthma COPD overlap syndrome (ACOS). ACOS was then divided to "primary asthma", "primary COPD" and "no primary disease" subgroups. A patient was classified into "primary asthma/COPD" subgroup if one of the diseases was diagnosed 10 years prior to the other. Figure 6 illustrates the division of patients into three diagnostic groups after re-evaluation and shows the proportions of ACOS subgroups.

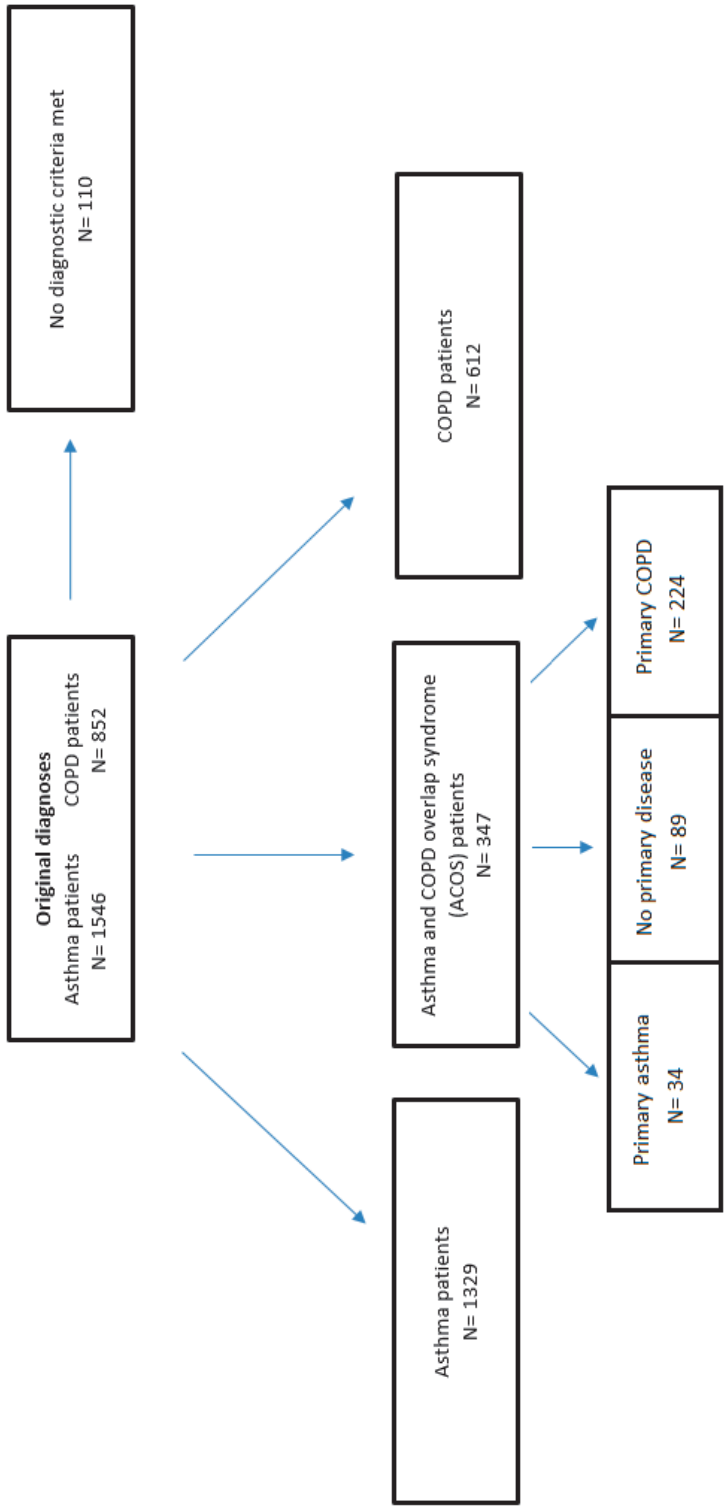

Figure 6. Re-evaluation and distribution of three diagnostic classes and subdivision of ACOS patients according to primary diagnosis.

4.1.8. Final data sets used in articles

Article I

In the first article regarding differences between asthma, COPD and ACOS patients, we analyzed only patients from the Helsinki region. After the re-evaluation of diagnoses we had 1755 patients with either an asthma, COPD, or ACOS diagnosis. One hundred and ninety nine patients were excluded because the data was unavailable at the time of the analyses, leaving the final study population of 1546 patients, of whom 70.1% were asthmatics, 15.3% COPD patients and 14.6% ACOS patients. The patients were excluded because of lack of spirometry data or if we were unable to find the patient's height from the medical records and thus unable to calculate the reference values for lung functions. For lung function classification Finnish criteria were used in articles II, III and IV. COPD and ACOS patients with no data on smoking duration or intensity were also excluded.

Article III and IV

In third and fourth articles the focus was on COPD patients and the data set was a subset of CAD cohort. This dataset included 739 patients with COPD or ACOS diagnosis with a primary diagnosis of COPD and it was used in several analyses and articles that were published before the articles III and IV were included into this thesis. The dataset was genotyped for the article IV. The genotyping success rate was 78% and because of that the number of patients used in analyses for article IV was 575.

Article II, COPEX study

During the research visit patients gave their permission to invite them to take part also in other studies. COPD exercise study (COPEX) was the first of the new studies. Its aim was to gather information about COPD patients' spontaneous exercise habits and physical activity. Information was also collected on the rehabilitation the patients had received because of their lung disease. COPEX study was performed in the year 2010. The COPEX questionnaire was sent to all COPD and ACOS patients who were participating in the CAD study at the time (Figure 4). After one reminder 719 patients were included in the study and the percent of participation was 87%. COPEX study outline is illustrated in Figure 7.

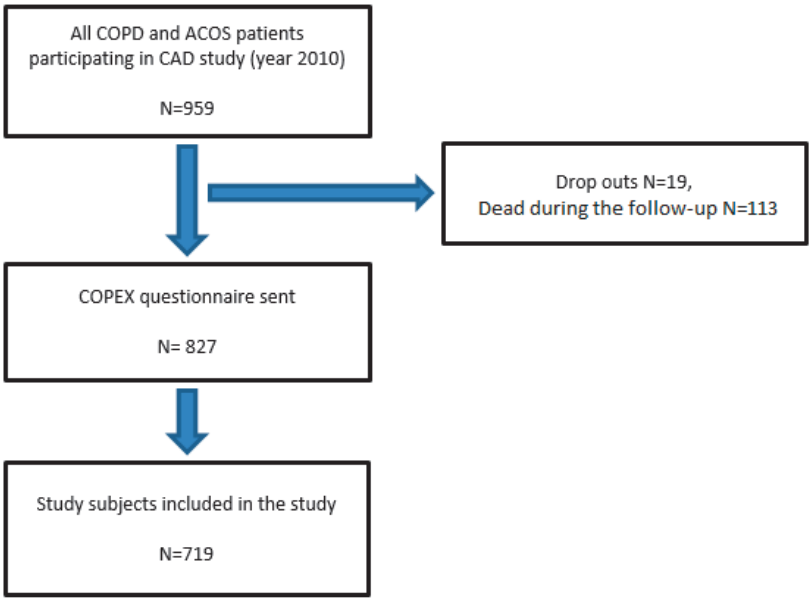

Figure 7. COPEX study outline and number of patients participating in the study.

4.1.9. Mortality during study

From the beginning of the study until the end of year 2013, 324 patients had died, 243 of the patients were from the Hospital District of Helsinki and Uusimaa and 81 patients from the Hospital Distric of Southwest Finland. Mortality percent of the whole CAD cohort varied between 1.0–1.6 % each year. Yearly mortality percent among asthmatics varied between 0.3–0.8%, among COPD patients 5.5–6.8% and ACOS patients 1.7–5.0%. Figure 8 shows each year's mortality rates in three main diagnostic categories. Research nurses checked patients' deaths from the national death registry before sending follow-up questionnaires.

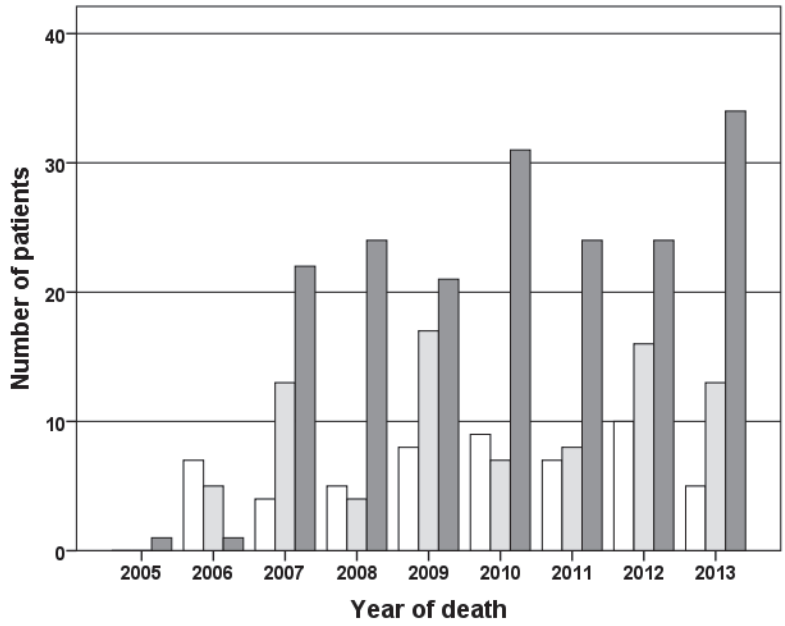

Figure 8. Annual death rate. Light bars: asthma patients, light grey bars: ACOS patients, dark grey bars: COPD patients.

4.1.10. Withdrawals during the study

Patients were free to withdraw from the study at any point without explanation. The number of patients withdrawn from the study until year 2014 was 77 patients (3.5%) in HUCH and 5 patients (1.7%) in TUCH. The reasons for withdrawal were not asked, systemically collected or analyzed but the main reasons patients gave for withdrawal were other chronical illnesses, notably dementia, which made patients unable to carry on in the study. The withdrawal percent was lower in Turku compared to the Helsinki region. One main reason is probably that the number of patients in Turku region was much smaller than in Helsinki and the same research nurse was personally in contact with all the patients several times during the recruitment process and the study.

4.2. Control data sets

4.2.1. Health2000

For the article IV, Health2000 study was used as a control population. (Aromaa A.) This study population was selected because it is a nationally representative sample of Finnish adults aged 30 or above and had information about genetic markers needed in

our analyses. A random subsample of the cohort consisting 1730 people was selected and used in the analyses.

4.2.2. ATBC study cohort

The results of the analyses of article IV were repeated in a sub cohort of ATBC study population. (The ATBC Cancer Prevention Study Group 1994) ATBC (Alfa-Tocopherol Beta-Carotene Cancer Prevention Study) study took place during years 1985–1993. The study population consisted of Finnish middle-aged men who smoked during recruitment. 1911 study subjects who had information on smoking history and genetic markers of interest were used in our study.

4.3. Study questionnaires

4.3.1. Research visit questionnaire

The research visit questionnaire was filled when patients were recruited in the study. The focus of the research questionnaire differed from the follow-up questionnaires considerably because the focus was more on asthma patients in the beginning of the study. The only similarity between the research visit questionnaire and follow-up questionnaires was that both included 15D and AQ20 HRQoL questionnaires. In the research visit patients were asked whether they had received compensation for their lung disease medication from the Social Institution of Insurance of Finland and whether they had purchased lung medication from abroad. The patients were also asked whether they had done PEF-measurements at home or had been instructed by medical personnel how to take medication.

All questionnaires were available in both Finnish and Swedish and were sent to the patients according to their mother tongue or preference.

4.3.2. Follow-up questionnaires

Questionnaires for the follow-up years 1 and 2 were identical. The patients were asked about their recent healthcare providers, their working status, profession, work ability, sick leaves and time they had spent at work during the last 12 months. Patients' smoking status, years of smoking initiation and cessation were inquired after, as well as the tobacco products used and quantities smoked. Patients' use of rescue medication (short acting bronchodilators) and medication for allergies were asked. 15D and AQ20 HRQoL questionnaires were also included in the follow-up questionnaires. The 4th year follow-up questionnaire included the same elements as follow-up 1 and 2 but the medication was asked in more detail. Both short and long acting bronchodilators and

use of oral corticosteroids were asked, as well as the dosages. Patients who still smoked were asked about their nicotine dependency with Fagerström test for nicotine dependency (FTND). The 6th year follow-up was similar to 4th year in other respects but FTND was replaced by the Modified British Medical Research Council (MMRC) dyspnea scale.

4.3.3. COPEX questionnaire

When planning the COPEX study, we designed the questionnaire with the presumption that if we knew more about patients' spontaneous exercise habits, we could use the data in the future to tailor more effective rehabilitation using those elements that were already present. This way the study may have direct clinical implications. The aim with the COPEX Questionnaire design was to create an easy to fill questionnaire that would cover not only the aspects of physical activity, but also estimates of patients' sensation of dyspnea, quality of life and received rehabilitation. When possible, the questions used in the questionnaire were taken from earlier, validated questionnaires. Questions 1–4 were from Finnish Health and Functional Capacity Study. (Aromaa, Koskinen 2004) In question 5, the most popular sports in Finland were listed to find out patients' exercise preferences. Question 6 was modified from an earlier study that examined barriers of physical activity among elderly people. (Rasinaho, Hirvensalo et al. 2007) Our study patients presented also severe stages of COPD with FEV1 values less than 30% of expected. Question 7 was designed to differentiate patients whose lung disease prevents them from exercising. Patients' subjective feeling of dyspnea was estimated with the MMRC dyspnea scale (question 8). (Bestall, Paul et al. 1999) We also wanted to have the patients' personal evaluation on how COPD had affected their exercise habits (question 9), and questioned the patients on their exercise habits in youth and on counseling and rehabilitation they have received because of COPD (questions 10–14). The response rate varied between 96–100% for individual questions.

4.4. Statistical methods

4.4.1. Basics

The IBM SPSS Statistics software (versions 16–20) was used in all statistical analyses. The data analysis started with the evaluation of the data. The distribution of values was checked for all variables, and outliers were detected and removed from the data before further analyses. The threshold for outliers was ±2 standard deviation (SD). After the removal of outliers, the normality of continuous variables was tested. P-value of 0.05 was used for level of significance in all analyses, in some analyses also p-value <0.001 was displayed. Confidence interval (CI) of 95% was used in all regression analyses.

4.4.2. Missing data

The variables that every patient needed to have to be included in the analyses were age, gender, pack years and spirometry test results (FEV1, FVC). The other variables used in the analyses and taken into multivariate regression models were found on at least 95% of the patients. If more values were missing or if an analysis could only be done to a subgroup of the study population due to missing data, it is stated in the article. This was the case for diffusion capacity values in article I and for FTND values in article III. All analyses in the fourth article were done for a subgroup of the cohort; only genotyped patients were included.

If any data was missing, it was not filled in. Instead, the slight decrease in the number of patients in the analyses was accepted. An exception was the FTND test in article III. To be able to calculate the value for the nicotine dependence, patients needed to answer all 6 questions of the FNDT questionnaire. Of the 155 patients who were still active smokers and thus eligible to answer the questionnaire, 140 provided a filled questionnaire, most of them with all answers and for the few who had maximum of two missing answers the questionnaires were taken into analysis after the missing values were filled in at random.

4.4.3. Asthma COPD overlap syndrome and HRQoL (article I)

In the first article we analyzed low lung specific HRQoL with logistic regression. The dependent variable was lung specific HRQoL score from AQ20 questionnaire that was divided into two classes. Low HRQoL was defined as mean score +1SD. Independent variables used for adjusted logistic regression model were gender, recruitment age, current smoking status, pack years, BMI, asthma/COPD/overlap group, duration of the airway disease, working status, cardiovascular diseases (coronary disease, cerebrovascular disease and claudication) and diabetes. Differences in independent variables between patients with low and normal HRQoL were analyzed with Mann-Whitney U-test for continuous variables and with chi-square test for categorical variables.

4.4.4. COPD patients' level of physical activity, exercise habits and rehabilitation (article II)

In the second article, patients were divided into active and inactive groups based on the history of physical activity levels. A logistic regression model was made with physical inactivity as a dependent variable and subjective sensation of dyspnea (MMRC classes), gender, age (as a continuous variable), smoking status, BMI, common comorbidities (CVD, diabetes, alcohol abuse and psychiatric conditions) and FEV1 Finnish criteria classes as independent variables. Differences in independent variables between active and inactive patients were analyzed with Mann-Whitney U-test for continuous variables and with chi-square test for categorical variables.

Interactions between lung function groups and exercise time, exercise activity, daily activity and mobility were tested with 2-way analysis of variance.

Spearman's rank correlation coefficient was used for analyzing correlations between patients' exercise activity, lung functions (FEV1% and diffusion capacity), MMRC scale, mobility score and both general and lung specific quality of life. Bonferroni correction was used for the analyses.

4.4.5. Smoking cessation's association to mortality and comorbidities (article III)

In article III two logistic regression models were tested, first with failure of smoking cessation as the dependent variable and second with mortality as the dependent variable. When analyzing factors affecting the failure of smoking cessation, the independent variables were gender, age, FEV1 Finnish criteria classes, pack years and comorbidities (CVD, diabetes, cancer, psychiatric disorders and alcohol abuse). Independent variables in the mortality model were gender, age, smoking cessation, FEV1 classes and same comorbidities as in the first logistic regression model.

Differences in independent variables between active and inactive patients were analyzed with Mann-Whitney U-test for continuous variables and with chi-square test and Fisher exact test for categorical variables.

Linear regression model was used in article III to analyze the FTND score. Independent variables into the model were gender, age, FEV1, alcohol abuse and comorbidities (psychiatric conditions, diabetes and CVD).

4.4.6. Genetic locus on nicotic acethylcholine receptor associates with increased mortality among smokers (article IV)

The association between COPD and genetic locus was analyzed in population control data set with PLINK whole genome association analysis toolset version 1.07.

Two logistic regression models were analyzed with CAD cohort. The dependent variables were heavy smoking (>40 pack years) and cancer prevalence, and the explanatory variable was additive model of SNP rs1051730. In the additive model, the variable is coded into three separate values: minor homozygote, heterozygote and major homozygote. Both crude and multivariate regression models were created and the multivariate model was adjusted for age, gender, smoking cessation, alcohol abuse, FEV1 Finnish criteria classes and for either cancer prevalence or pack years depending on the dependent variable.

rs1051730 SNP's association to mortality was analyzed with Cox regression. Apart from SNP, the multivariate model was adjusted with gender, pack years, smoking cessation, BMI, FEV1 Finnish criteria classes and comorbidities (CVD, diabetes, cancer, psychiatric diseases and alcohol abuse).

Cox regression for mortality and logistic regression for heavy smoking were repeated in cohort consisting of male smokers. There was less data on male smoker cohort so both logistic and Cox regression multivariate models were adjusted with only pack years, smoking cessation, BMI, alcohol abuse and FEV1 classes.

Kaplan-Meier analysis with log rank test was used for analysis of survival during the follow-up and Kaplan-Meier curves were drawn for both CAD and male smoker cohorts. The dependent variable was additive model of rs1051730 SNP. Interactions between the SNP and explanatory variables were tested with a likelihood ratio test.

Table 4. Summary of used statistical methods.

Article	Statistical method	Use of the method
I, II,III, IV	Logistic regression	to find out OR of independent variables on categorical (dichotomous) dependent variable
I, II, III	Mann-Whitney U-test	to analyze statistically significant difference between two independent continuous variables
I, II, III	Chi-square test	to analyze statistically significant difference between two independent categorical variables
II	ANOVA	to analyze statistically significant difference between mean values of continuous variables
II	Spearman's rank correlation	to analyze the correlation between two continuous variables
III	Linear regression	to analyze the effects of independent variables on continuous dependent variable
IV	Cox regression	to find out HR of independent variables on categorical dependent variable
IV	Kaplan-Meyer	to illustrate and analyze the difference in the speed to end point event (mortality) in different groups

4.5. DNA extraction and genotyping

During the research visit (years 2005–2007) a 7 ml citrate blood sample was collected from all study subjects. The samples were stored in -20°C before DNA extraction. The DNA was extracted with the Qiagen, QIAamp Blood Maxi kit in Iceland in collaboration with deCODE genetics in 2009. During years 2009 and 2010 all samples were diluted to concentrations of 10–80 ng/ml in TE-buffer and stored in -20°C. Study subjects with COPD as primary or secondary diagnosis (COPD or ACOS patients) were genotyped in Iceland for 94 candidate gene SNPs that had the most promising association to COPD in the Icelandic population. Illumina method was used for genotyping.

4.6. Ethical aspects

The design of the study was approved by the Coordinating Ethics Committee of Helsinki and Uusimaa Hospital District (number of research permit: 125/E0/2004). Universities of Helsinki and Turku granted a permission to conduct the study. The permission was applied to and re-evaluated every year. COPEX study was evaluated independently (number of research permit 319/13/03/00/09).

An informed consent was collected from all patients who took part in the study. Because of the complexity of the study design, a specially trained research nurse explained the study design to every patient individually and made sure that the patient understood the nature of the study.

In the beginning of the study, patients' medical records were collected from different health care providers. This data was then de-identified by research nurses and scanned to digital form. The original medical records and digital copies were kept locked in the research center. Researchers kept the de-identified medical history data in the research center on a computer that was not connected to Internet or other devices. The data was also extracted with this computer, and only after that was the coded data moved to other devices to make sure that patient data was safe.

Good clinical practice was followed throughout the study. A monitor outside the research group monitored all follow-up questionnaires. Returned questionnaires without signatures or with some other discrepancies were removed after every round of follow-up questionnaires, and data they held was not digitalized or included in the study data. The follow-up data was included in the analyses only after the manager of the research center had signed all returned questionnaires, they were monitored and the monitor had ensured that all needed changes and removals of data were done.

Only research nurses had access to patients' identifying data; the researchers knew only patients' year of birth, gender and region (Helsinki or Turku).

The patients could withdraw from the study at any point without an explanation. If a patient was withdrawn from the study before DNA extraction, his/her blood sample was excluded from the DNA extraction and (s)he wasn't included in the genetic analyses. If a patient was in such a poor health that (s)he couldn't sign the withdrawal application, their family informed the research nurse. The withdrawal from study was done after the monitor had approved it.

Patients were also informed about the latest progress of the research with every follow-up questionnaire.

5. RESULTS

5.1. Description of CAD cohort

Basic demographic data on CAD cohort is displayed in table 4. Of 2288 patients approximately 58% were asthmatics, 27% COPD patients and the remaining 15% ACOS patients. Over two third of asthmatics were women, whereas only one third of COPD patients were women. Gender distribution of ACOS patients was more or less equal.

COPD patients were diagnosed at an older age than asthmatics, but the duration of the disease was shorter than among asthma patients. ACOS patients' diagnosis age and duration of disease were in between those of asthma and COPD patients'. Thirty two percent of COPD patients had died at the end of year 2013, when the follow-up time had been 6–8 years. Mortality among ACOS patients during the follow-up was 26% and among asthmatics 4%. Over 50% of asthmatics had never smoked, and only 11% are current smokers. The portion of COPD and ACOS patients who have managed to quit smoking is encouraging; over 50% in both disease groups. Differences in pack years between asthmatics, COPD and ACOS patients were large and reflect the smoking status data.

On average, asthmatics had normal lung functions. Mean pre-bronchodilation FEV1 in COPD patients was 59% with FEV1/FVC ratio of 0.65, which is well under the diagnostic limit of 0.70. Even though a modest response on FEV1 and FVC values was noticed after bronchodilation, the FEV1/FVC ratio did not change. In ACOS patients the response to bronchodilators was larger than in COPD patients.

All chronic comorbidities listed in table 4 were more common among COPD patients than asthmatics. In asthmatics, the most common comorbidity was psychiatric condition (19%), in COPD patients, cardiovascular diseases (28%), with psychiatric conditions following closely (24%). Alcohol abuse was very common among COPD patients (14%) and ACOS patients (15%), whereas only 3% of asthmatics abused alcohol. Surprisingly, cancer prevalence was a bit higher in asthmatics than in COPD or ACOS patients. Asthma patients had a better general and lung specific HRQoL than COPD or ACOS patients. ACOS patients' HRQoL scores were especially low.

Table 5. CAD cohort descriptive data.

	Asthma	COPD	ACOS
N (%)	1329 (58.1%)	612 (26.7%)	347 (15.2%)
Men (N,%)	347 (26.1%)	400 (65.4%)	184 (53.0%)
Women (N,%)	982 (73.9%)	212 (34.6%)	163 (47.0%)
Age at recruitment (mean, SD)	53.8 (12.5)	64.2 (6.9)	62.2 (7.1)
Age at diagnosis (mean, SD)	42.8 (16.0)	58.7 (7.2)	53.4 (11.0)
Duration of disease at recruitment, years (mean, SD)	14.8 (11.7)	8.0 (4.2)	11.9 (9.6)
Dead during follow-up (N, %)	57 (4%)	198 (32%)	91 (26%)
Smoking status during recruitment (N, %)			
current smoker	134 (10.9%)	210 (39.8%)	136 (44.9%)
ex-smoker	427 (34.6%)	298 (56.4%)	155 (51.2%)
never smoker	673 (54.5%)	20 (3.3%)	12 (4.0%)
Pack years (mean, SD)	5.9 (11.9)	43.3 (25.8)	37.8 (21.7)
Lung functions			
Baseline (mean, SD)			
FEV1 % of expected	86% (17)	59% (19)	60% (19)
FVC % of expected	90% (15)	73% (18)	76% (19)
FEV1/FVC	0.78 (9)	0.65 (14)	0.64 (14)
After bronchodilation (mean, SD)			
FEV1 % of expected	87% (16)	62% (18)	65% (19)
FVC % of expected	90% (15)	77% (17)	82% (17)
FEV1/FVC	0.79 (9)	0.65 (14)	0.64 (14)
Comorbidities (N, %)			
CVD	133 (10.0%)	169 (27.6%)	88 (25.4%)
Diabetes	90 (6.8%)	96 (15.7%)	43 (12.4%)
Cancer	104 (7.9%)	37 (6.1%)	22 (6.3%)
Alcohol abuse	44 (3.3%)	88 (14.4%)	50 (14.8%)
Psychiatric condition requiring medication	248 (18.8%)	148 (24.2%)	77 (22.2%)
15D score (mean, SD)	0.86 (0.10)	0.79 (0.11)	0.79 (0.10)
AQ20 score (mean, SD)	7.0 (4.7)	7.9 (4.9)	9.4 (5.0)
BMI (mean, SD)	27.2 (5.5)	26.6 (5.2)	26.9 (5.8)

CVD: coronary heart disease, cerebrovascular disease and claudication.

5.2. Features of patients with asthma and COPD overlap syndrome (ACOS)

In article I the differences between asthma, COPD and ACOS patients were analyzed. The analyses were performed in a subset of CAD cohort; patients from the Helsinki region were included in the study, N=1546. In this subset of CAD cohort, the prevalence of ACOS patients was 14.5%. 10.2% of patients, whose initial diagnosis was asthma, were classified as ACOS patients. Among COPD patients the percent of ACOS patients was 22.2%.

COPD patients had received the diagnosis on average seven years before the recruitment. Asthma patients were diagnosed 11 years before the recruitment, even though asthmatics are on average 11 years younger than COPD patients, which illustrates the typical different age of onset of these diseases. Patients with both diagnoses (ACOS patients) had received their first diagnosis approximately eight years before recruitment to the study.

ACOS patients' age, gender distribution, duration of disease and number of pack years differed significantly from both asthma and COPD patients and were in between the values of asthma and COPD patients. Lung specific quality of life was, on the other hand, significantly lower among ACOS patients compared to either asthma or COPD patients: mean AQ20 score among ACOS patients was 8.8, among COPD patients 7.4 and among asthmatics 6.8. ACOS patients' work ability index (which measures patients subjective work ability compared to their personal best on a scale from 1 (personal worst) to 10 (personal best)) was also significantly lower among ACOS patients compared to asthmatics and COPD patients: mean work ability index among ACOS patients was 6.9, among COPD patients 7.1 and among asthmatics 8.2.

Multinomial logistic regression showed that independent factors contributing to low lung specific HRQoL were ACOS diagnosis and, to lesser extent, COPD diagnosis when compared to asthma (table 5). Also BMI ≥ 30, duration of the disease for over 10 years, being on disability pension and comorbid cardiovascular disease had an independent explanatory value to low HRQoL.

Table 6. Multinomial logistic regression model for low lung specific HRQoL.

	N of patients	**% of patients with low HRQoL**	**Adjusted OR**	**95% CI**
Gender				
Male	495	20.6	1.0	
Female	946	23.3	1.5	1.1-2.1 *
BMI				
<19	41	22.0	0.8	0.3-2.0
≥19 and <30	1006	19.3	1.0	1.0
≥30	380	30.3	1.6	1.2-2.2 **
Disease group				
Asthma only	1024	19.3	1.0	
COPD only	212	27.4	1.9	1.2-3.0 *
Overlap syndrome	205	32.2	1.7	1.0-2.9 *
Duration of the airway disease				
<10 years	833	19.0	1.0	
≥ 10 years	607	27.0	1.6	1.2-2.2 **
Work status				
In working life	741	15.1	1.0	
On old age pension	437	21.1	1.1	0.7-1.6
On disability pension	233	46.4	3.2	2.1-4.8 **
CVD	587	29.8	1.4	1.1-2.0 *

Significant associations shown. The model is adjusted with recruitment age, pack years, current smoking and diabetes. * $p<0.05$, ** $p<0.01$. Modified from Kauppi et al (Kauppi, Kupiainen et al. 2011).

In our study population ACOS patients' mortality rate was consistently between those of asthma and COPD patients, varying between 1.7–5.0 % each year (asthmatics' 0.3–0.8% and COPD patients' 5.5–6.8% each year).

5.3. Physical activity and rehabilitation of COPD patients

To study the physical activity and exercise levels of Finnish COPD patients, all COPD and ACOS patients who were still actively participating in CAD study in the year 2010 were invited to take part in COPEX study. Patients included in the study (N=719) were divided to physically active and inactive groups according to the American Heart Association's definition for physical activity: exercise at least 2–3 times a week at least 30 minutes at a time throughout the year with an intensity causing shortness of breath and perspiration. (Haskell, Lee et al. 2007) 49% of patients were physically active year round. The average time of exercise among physically active patients was 135 minutes per week and 29 minutes among inactive patients. 120 (34%) of inactive patients did not do any exercise. The questionnaire covered also the level of daily life activities, e.g. household chores, shopping and gardening, the presumption being that patients with a more severe disease would spend time with them instead of more strenuous exercise. The mean time used in daily life activities was 350 minutes per week among physically active patients and 280 minutes among inactive. 15% of the inactive patients reported that they spent less than an hour a week in daily life activities. The time used with daily life activities was very similar among physically active patients regardless of severity of obstruction (minutes used per week varying from 360–440 minutes) but among inactive patients poor lung functions associated with reduced time used at daily life activities.

Physically active patients did not differ from physically inactive ones when comparing age, gender, BMI or comorbidities (hypertension, cardiovascular diseases, diabetes, psychiatric conditions, alcohol abuse). Lung functions (FEV1 and FVC) and diffusion capacity values differed significantly between physically active and inactive patients (FEV1 63% and 58% of predicted, FVC 78% and 73% of predicted, DLCOcVA 82% and 75% of predicted, respectively). Also the number of patients that had quit smoking differed significantly among active and inactive patients (66% vs. 57%, respectively).

To find out what differentiated active and inactive patients with similar lung functions, the patients were divided into Finnish criteria groups according to their FEV1 value (normal values: FEV1 >80% N=102, mild obstruction: FEV1 65–80% N=187, moderate obstruction: FEV1 40–65% N=286, severe obstruction: FEV1 <40% N=106). (Viljanen, Halttunen et al. 1982) 60% of patients with normal FEV1 values were physically active; among patients with mild or moderate obstruction the portion was approximately 50%. Patients with severe obstruction (FEV1 <40%) were understandably less active than other patients, but even among them 33% of patients reported of being physically active year round. Besides lung functions, also seasons

affect physical activity among CAD patients. Among patients with mild to severe obstruction the time used for physical exercise was significantly lower during winter (figure 9).

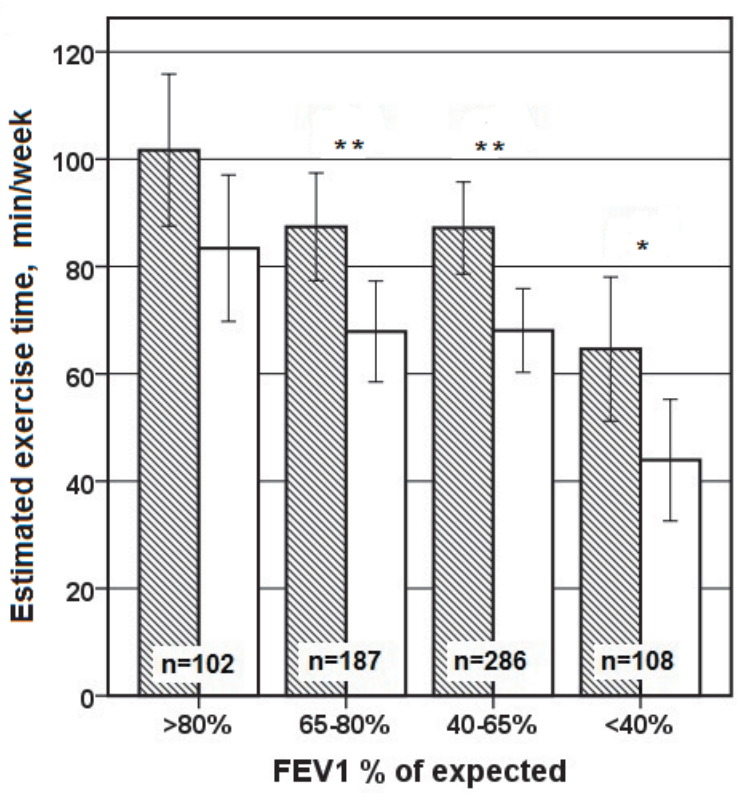

Figure 9. COPD patients' exercise habits depend on season and level of obstruction. Grey bars: spring, summer and autumn, white bars: winter. *p<0.05, ** p<0.01. Modified from Katajisto et al. (Katajisto, Kupiainen et al. 2012).

Subjective sensation of dyspnea was measured with Modified Medical Research Council's (MMRC) dyspnea scale. MMRC scale goes from 0 (not troubled with breathlessness except with strenuous exercise) to 4 (too breathless to leave the house or breathless when dressing). Physically active patients' mean MMRC value was 1.36, compared to inactive patients' mean value of 1.93 (p>0.01). Subjective sensation of dyspnea increased moderately among physically active patients when lung functions got poorer; mean MMRC values were 1.02, 1.34, 1.38 and 1.76 with normal, mild, moderate and severe obstruction, respectively. With physically inactive patients the sensation of dyspnea was more pronounced in all lung function groups and increased more rapidly; mean MMRC values were 1.17, 1.60, 2.04 and 2.56 with increasing level of obstruction. The difference in subjective sensation of dyspnea between active and inactive patients was significant among patients with moderate and severe obstruction (p<0.01). Figure 10.

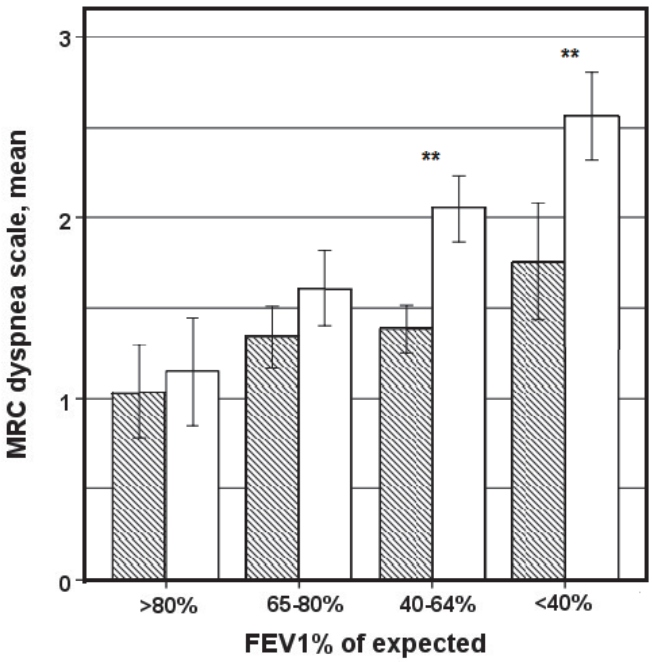

Figure 10. The mean values of MMRC dyspnea scale in different levels of obstruction. Grey bars: physically active patients, white bars: physically inactive patients. ** p<0.01. Modified from Katajisto et al. (Katajisto, Kupiainen et al. 2012).

Multivariate logistic regression adjusted with gender, age, smoking status, BMI and comorbidities found that subjective sensation of dyspnea was the strongest factor explaining physical inactivity (OR 7.3 (CI 95% 3.2–16.6) for MMRC class 3 and OR 11.5 (CI 95% 3.6–37.2) for MMRC class 4). Lung functions explained inactivity only when FEV1 was below <40% (OR 3.5, (CI 95% 1.8–6.9)) and current smoking had weak explanatory value on inactivity (OR 1.5 (CI 95% 1.0–2.4)).

Only fifteen percent of patients had received pulmonary rehabilitation and 36% had received guidance for training from health care personnel. Physically active patients had received training guidance from medical doctor more often than inactive patients (18% vs 11%, p=0.02), but both patient groups had received the same amount of guidance by nurses and/or physiotherapists. There was no difference in attendance to pulmonary rehabilitation among physically active and inactive patients. Inactive patients were more interested in receiving rehabilitation in the future (62%) compared to active patients (54%) (p<0.05).

Both general and lung-specific HRQoL are correlated to physical activity and daily life activity levels with rather weak but statistically significant correlations. MMRC dyspnea scale and mobility score had the strongest correlations with general 15D score and lung-specific AQ20 score. When analyzing active and inactive patients separately, active and inactive patients' AQ20 scores were 7.5 and 9.0 (p>0.01). There was no difference in the general 15D score between active and inactive patients.

COPEX questionnaire was sent to all COPD and ACOS patients who were alive in spring 2010. When the analyses were done on years 2010 and 2011 we did not have any mortality data on COPEX patients. At the moment we have complete mortality data from years 2011–2013. The number of deaths were 25 patients (3.5%), 32 (4.5%) and 43 (6%) on years 2011, 2012 and 2013, respectively. 17% of physically active and 22% of inactive patients had died during that time. The difference wasn't statistically significant.

5.4. Smoking cessation among COPD patients

Smoking cessation and factors related to it were analyzed in a subcohort consisting of all COPD patients and ACOS patients that had a primary diagnosis of COPD (N=739). All patients of this subcohort have a long smoking history (mean 41 pack years). 59% of the patients had quit smoking before recruitment to the study, 28% before receiving the COPD diagnosis (early quitters) and 31% after that (late quitters). Men were more likely to quit smoking before the COPD diagnosis than women; at the time of diagnosis 80% of women smoked compared to 68% of men. Interestingly, quitters had more pack years than non-quitters. They were also older and their lung functions were lower than those of non-quitters. Regardless of those facts, quitters had a better general quality of life (15D score among quitters 0.80 and among non-quitters 0.78, $p<0.05$). Alcohol abuse and psychiatric disorders were more common among non-quitters, 12% of quitters abused alcohol and 18% had a psychiatric condition, compared to 27% of non-quitters with alcohol abuse and 34% with a psychiatric condition ($p<0.01$ for both differences). Cardiovascular disease were more common among quitters (33%) than non-quitters (25%) ($p=0.02$), and the number of patients with CVD was especially high among early quitters. Although non-quitters were younger, their mortality rate was higher than quitters'. In multivariate logistic regression model for failure in smoking cessation, alcohol abuse and psychiatric conditions increased the risk significantly (OR 2.1 (CI 95% 1.4–3.3) and OR 1.83 (CI 95% 1.2–2.7) respectively). Alcohol abuse and psychiatric conditions coexist, especially among non-quitters, among whom the portion of patients with both conditions was 14%. Among quitters these two conditions coexisted in 3% of patients. Aging increased the chance of quitting smoking, but gender, lung functions, pack years, CVD, diabetes or cancer did not affect the risk of failing in smoking cessation.

Mortality among non-quitters was 21% and among quitters 12% ($p<0.01$). In multivariate regression model failure in smoking cessation, aging, alcohol abuse, cancer and moderate to severe obstruction increased the risk of mortality (Table 6).

Nicotine dependency was evaluated with Fagerström test for nicotine dependency (FTND) as a part of the 4th year follow-up questionnaire. FTND score ranges from 0–10. The mean score among patients who still smoked was 4.3 (SD 2.4). In linear regression model adjusted with age, gender, lung functions, CVD, diabetes, cancer, alcohol abuse and psychiatric conditions only alcohol abuse and psychiatric conditions

associated with the level of nicotine dependence; beta value for alcohol abuse was 0.26, p<0.01 and for psychiatric conditions 0.18, p<0.05.

Table 7. Multivariate logistic model for failing in smoking cessation.

Variables	Patient N= 739	Deceased %	Adjusted OR	95% CI
Aging by one year			1.07	1.03–1.11
Smoking cessation				
yes	426	12.2	1.00	
no	299	20.7	2.50	1.55–4.03 **
Alcohol abuse				
no	584	13.7	1.00	
yes	128	24.2	2.03	1.14–3.61 *
Cancer				
no	649	13.7	1.00	
yes	74	32.4	3.08	1.70–5.59 **
FEV1% of predicted				
>80%	87	5.7	1.00	
80–65%	191	9.9	2.12	0.68–6.67
65–40%	306	16.5	3.76	1.27–11.15 *
<40%	136	28.7	8.77	2.88–26.68 **

Significant associations shown. Model adjusted with gender, CVD, diabetes and psychiatric conditions. *p<0.05, **p<0.01. Modified from Kupiainen et al. (Kupiainen, Kinnula et al. 2012).

5.5. rs1051730 effect on COPD patients and male smokers

COPD patients' DNA was extracted in Iceland. 94 candidate gene SNPs that showed most promising association to COPD in the Icelandic population were genotyped after DNA extraction. The genotyping was done to 575 CAD cohort patients. rs1051730 SNP is located in nicotinic acethycholine receptor gene cluster CHRNA3/5 in chromosome 15 and had the strongest association to COPD when tested in Finnish cohorts. SNP's minor allele frequency (MAF) was 0.35 in general Finnish adult population and 0.38 in CAD cohort, OR 1.4 (CI 95% 1.2–1.7), p= 3.2x10-5.

rs1051730 SNP's associations to heavy smoking (>40 pack years), prevalence of any type of cancer and mortality were analyzed with logistic and Cox regression. Additive model of the SNP was used (major homozygote (wild type) GG, heterozygote AG and minor homozygote AA). The analyses for heavy smoking and mortality were repeated in ATBC subcohort consisting of male smokers (N=1911). Unfortunately data on cancer prevalence, lung functions and comorbidities were missing in male smoker cohort. Variables used in analyses are summarized in table 8.

Table 8. Variables used in CAD cohort and male smoker cohort.

Variables used in CAD cohort	Variables used in male smoker cohort
rs1051730 SNP	rs1051730 SNP
Mortality	Mortality
Age	Age
Pack years	Pack years
Smoking cessation	Smoking cessation
Alcohol abuse	Alcohol abuse
BMI	BMI
Gender	
FEV1 Finnish criteria classes	
Cancer	
Cardiovascular disease	
Diabetes	
Psychiatric condition	

In CAD cohort, history of heavy smoking did not associate with rs1051730. Alcohol abuse, moderate to severe obstruction of airways (FEV1 <65%), current smoking and age increased the risk of heavy smoking. Female gender decreased the risk significantly. In male smoker cohort, however, both rs1051730 heterozygote and minor homozygote increased the risk for heavy smoking (OR 1.3 (CI 95% 1.1–1.6), $p<0.01$ and OR 1.4 (CI 95% 1.0–1.9) $p<0.05$, respectively), as did alcohol abuse, current smoking and aging.

Of all tested variables, only aging (OR 1.1 (CI 95% 1.0–1.1) $p<0.01$) and rs1051730 minor homozygote (OR 2.3 (CI 95% 1.0–5.1) $p<0.05$) increased the risk of any type of cancer in CAD cohort.

Associations between all-cause mortality and rs1051730 were tested in both cohorts with Cox regression. First, the association was analyzed with univariate regression and afterwards also multivariate model was done, adjusted with independent variables listed in table 9. The adjustment done for multivariate model affected outcomes very little. In CAD cohort, minor homozygote of rs1051730 increased the risk of all-cause mortality (HR 2.2 (CI 95% 1.2–3.8), $p<0.01$). Alcohol abuse, FEV1<40%, and psychiatric conditions had also independent explanatory value. Smoking cessation and female gender decreased the risk significantly. In male smoker cohort both heterozygote and minor homozygote increased the risk of mortality significantly (OR 1.2 (CI 95% 1.0–1.3), $p<0.05$ and OR 1.3 (CI 95% 1.1–1.5), $p<0.01$, respectively). Pack years >40, obesity, current smoking and alcohol abuse also increased the risk (table 9).

Table 9. Risk factor for mortality in CAD and male smoker cohort.

	CAD cohort		**Male smoker cohort**	
	Univariate HR (CI)	**Multivariate HR (CI)**	**Univariate HR (CI)**	**Multivariate HR (CI)**
SNP, additive model				
major homozygote	1.0	1.0	1.0	1.0
heterozygote	1.2 (0.7–1.9)	1.1 (0.7–1.8)	1.2 (1.0–1.3)*	1.2 (1.0–1.3)**
minor homozygote	2.2 (1.2–3.8)**	2.6 (1.4–4.9)**	1.3 (1.1–1.5)**	1.2 (1.0–1.5)*
Men	1.0	1.0		
Women	0.6 (0.4–0.9)*	0.8 (0.5–1.3)		
Pack years >40 yrs	1.3 (0.9–1.9)	1.1 (0.7–1.8)	1.2 (1.1–1.4)**	1.1 (1.0–1.3)*
Smoking cessation	0.4 (0.3–0.6)**	0.5 (0.3–0.8)**	0.8 (0.7–0.9)**	0.8 (0.7–1.0)**
BMI				
Normal (≤25)	1.0	1.0	1.0	1.0
25–30	0.9 (0.6–1.3)	1.2 (0.7–2.2)	0.9 (0.8–1.1)	1.0 (0.9–1.1)
>30	0.9 (0.6–1.4)	1.4 (0.8–2.7)	1.2 (1.0–1.4)*	1.2 (1.0–1.4)*
Alcohol abuse	2.8 (1.9–4.2)**	3.4 (2.0–5.9)**	1.7 (1.4–2.0)**	1.6 (1.4–1.9)**
Psychiatric disease	1.6 (1.1–2.3)*	1.4 (0.8–2.5)		
Cancer	1.5 (0.9–2.2)	1.7 (1.0–3.0)*		
FEV1% of predicted				
>80	1.0	1.0		
65–80	1.4 (0.5–3.8)	1.2 (0.4–4.0)		
65-40	2.0 (0.8–4.9)	2.4 (0.8–7.1)		
<40	3.4 (1.3–8.6)*	5.3 (1.8–15.6)**		

Significant associations shown. CAD multivariate model adjusted for CVD and diabetes. * $p<0.05$, ** $p<0.01$. Modified from Kupiainen et al, manuscript in publication.

Overall survival was tested with Kaplan-Meier analysis and it confirmed the poorer survival of minor homozygotes (AA) compared to heterozygotes (AG) or major homozygotes (GG) (Figure 11). Table 10 summarizes the association between rs1051730 and dependent variables in CAD and male smoker cohort.

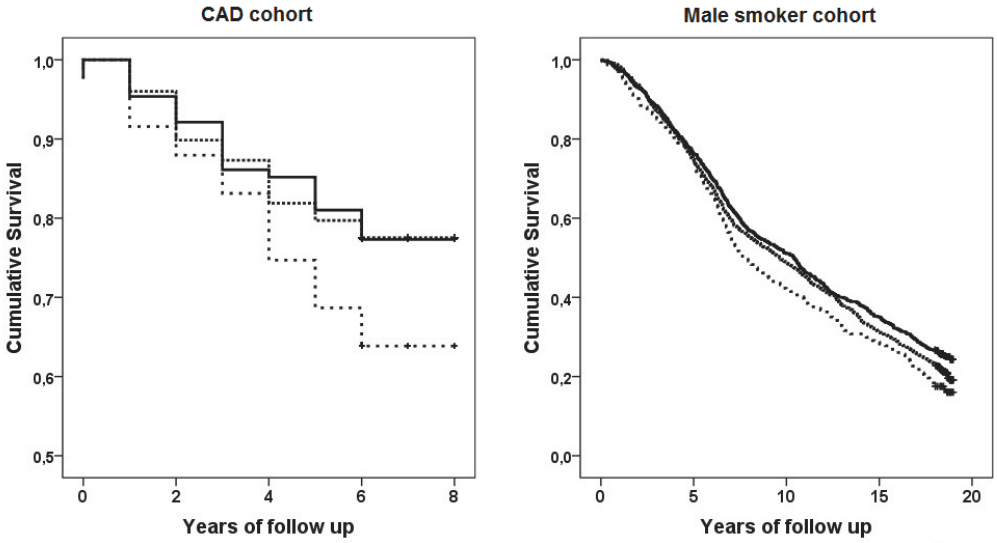

Figure 11. Kaplan-Meier curves of overall survival in CAD cohort and male smoker cohort. Solid line: major homozygote (GG), dashed line: heterozygote (GA), dotted line: minor homozygote (AA). Modified from Kupiainen et al, manuscript in publication.

Table 10. Summary of outcomes in logistic (heavy smoking, cancer) and Cox (mortality) regression in CAD cohort and male smoker cohort. Additive model of rs105730 SNP as an independent variable.

	CAD cohort	**Male smokers**
Mortality	minor homozygote had significant association	heterozygote and minor homozygote had significant association
History of heavy smoking (>40 pack years)	no association found	heterozygote and minor homozygote had significant association
Cancer prevalence	minor homozygote had significant association	not tested

6. DISCUSSION

6.1. ACOS and other factors associated with poor HRQoL

ACOS patients' lung-specific HRQoL is significantly poorer than that of asthma or COPD patients. This finding is in accordance with other studies that have emphasized the challenges in disease management in ACOS. (Barrecheguren, Esquinas et al. 2015) In addition to HRQoL, in our study the ACOS patients had the lowest work ability index and the highest number of long sick leaves; 64% of ACOS patients' sick leaves were >10 days long. Although ACOS patients were on average three years younger than COPD patients, there was no significant difference in the number of patients on pension. 27.5% of ACOS patients were in disability pension compared to 26% of COPD patients. In old age pension, the percent of COPD patients was little higher. The combination of poor HRQoL and challenges with coping at work, even though patients have better lung functions and less comorbidities than COPD patients, draws a picture of a sensitive patient group that has problems managing their disease and would probably benefit from more attentive care, especially when keeping in mind that at the moment ACOS patients' use of health care services is extensive. (Andersen, Lampela et al. 2013) New Finnish current care guidelines cover also the treatment of ACOS patients, but because ACOS patients are usually excluded from drug trials, evidence-based treatment recommendation cannot be given. (Kankaanranta, Harju et al. 2015)

There are several factors that affect HRQoL in COPD patients. We found that psychiatric conditions, especially anxiety and depression, have a strong negative effect on HRQoL. This is in accordance with earlier studies. (Blakemore, Dickens et al. 2014) Also subjective sensation of dyspnea is a strong marker for poor HRQoL both in our study and in literature. (Tsiligianni, Kocks et al. 2011) The characteristics that do not associate with poor HRQoL are also interesting. Both in literature and in our own study we found that e.g. age does not affect HRQoL scores, and most of the comorbidities also play a surprisingly small role, as does BMI. Airflow limitation starts to affect HRQoL only when it's severe. (Tsiligianni, Kocks et al. 2011)

Some studies have reported increased mortality in ACOS patients compared to asthma or COPD alone (Diaz-Guzman, Khosravi et al. 2011). CAD cohort's ACOS patients are on average 3 years younger than COPD patients but older than asthmatics. Because of this, differences in mortality rates in different diagnostic groups can result, at least partially, from the age difference, and conclusions on the effect of lung disease diagnosis or comorbidities are hard to draw. It would be very interesting to see what the mortality rates would be if there was no age difference between the groups.

6.2. Factors affecting physical activity in COPD patients

The number of physically active patients in CAD cohort was relatively high (50%). In other studies the percentage of physically active COPD patients varies between 30–60%. (Spruit 2014, Watz, Waschki et al. 2009) The difference in numbers can rise from several reasons. First, patients may exaggerate their exercise levels. Exaggeration and underestimation are common problems in questionnaire-based studies, and researchers need to be aware of this when interpreting the results. Second, the definition for physical activity used in our study was not as strict as in some other studies published in recent years. We used the American Heart Association's definition for physical activity (exercise at least 2–3 times a week at least 30 minutes at a time throughout the year with an intensity causing shortness of breath and perspiration). (Haskell, Lee et al. 2007) In the stricter definition, a physically active patient needs to exercise 5 times a week; either 30 minutes with lower intensity or 20 minutes with higher intensity or at intensive spurts with the duration of at least 10 minutes. (Watz, Pitta et al. 2014) We did not use this definition, as it would have made the questionnaire more complicated for the patients and the interpretation more difficult for us.

It is clear after our analyses that subjective sensation of dyspnea is a key player preventing COPD patients from physical activity. When analyzing the reasons that prevent patients from exercising, they could roughly be divided into three categories. First, clinical symptoms: sensation of dyspnea, pain or limitations brought by other chronic diseases. Second, motivational barriers and lack of information or skills: patient is not interested in exercise or it feels uncomfortable, (s)he doesn't know how to exercise or is afraid of hurting her/himself. Third, practical aspects: facilities for exercising are too far or too expensive, transportation is difficult or the weather is too bad for exercising. Dyspnea was by far the most common reason that restricted patients from exercising, 67% of patients reported it as a reason for exercise restriction. This is in accordance with the outcome of logistic regression model which stated that subjective sensation of dyspnea measured by MMRC scale is the strongest nominator for physical inactivity. Also other chronic diseases, pain and the fact that exercise felt uncomfortable were common reasons for not exercising (42%, 36% and 20% respectively).

MMRC scale has been shown to be a reliable nominator for physical activity levels in other studies too. (Watz, Waschki et al. 2009) It is a short and a practical way to estimate the level of dyspnea and could be used more in clinical settings and especially when directing patients to rehabilitation. (Watz, Waschki et al. 2009) Encouragement to exercise is crucial because it increases quality of life and decreases exacerbations and mortality. (Watz, Pitta et al. 2014)

It was surprising to find out that in a multivariate logistic regression analysis comorbidities, age, gender or BMI did not associate with physical activity and that the level of obstruction started to have an effect only in the late stages of the disease. It was very interesting that pulmonary rehabilitation did not seem to result in long-term changes of patients' exercise routines. Active patients had received guidance on

physical activity and training given by medical doctor more often than inactive ones. It seems unlikely that doctors would have given guidance especially to patients that are already physically active, so we can postulate that guidance by a doctor does motivate patients to exercise more regularly. Unfortunately, the guidance given by a nurse or physiotherapist did not have a similar effect. It is possible that patients see a doctor as a stronger authority and that has a bigger effect on their behavior. Approximately 15% of both active and inactive patients had received pulmonary rehabilitation. The result is surprising because the aim of the rehabilitation is to activate patients and establish long-term changes in their life style that promote health. It seems that these long-term goals were not reached with COPD patients. Patients were, however, interested in pulmonary rehabilitation. Inactive patients seem to realize their need for life style changes; 62% of inactive patients were interested in rehabilitation compared to 54% of active patients. In Finland the long-term outcomes of pulmonary rehabilitation have not been systemically analysed before this study.

COPEX questionnaire was sent to all patients with a COPD diagnosis. Among them were also ACOS patients with primary diagnosis of COPD. ACOS patients' HRQoL is lower than COPD patients' and their bronchial obstruction is more reversible. They also have more bronchial hyper-responsiveness than COPD patients. MMRC scale, measuring patients' subjective sensation of dyspnea, did not, however, find a difference between COPD and ACOS patients. It seems that other factors apart from the diagnosis have a stronger effect on experiencing dyspnea. Level of physical activity did not differ significantly in COPD and ACOS patients.

COPD patients' responsiveness to medication varies and insufficient medication is common. (Ingebrigtsen, Marott et al. 2013) Undertreatment is very likely one of the reasons for pronounced sensation of dyspnea. Frequent exacerbations also accelerate the decrease of lung functions and increase the sensation of dyspnea. (Mullerova, Lu et al. 2014) Unfortunately we did not have data on exacerbations in our cohort. COPD patients suffer from multimorbidity and have a high prevalence of depression and other psychiatric conditions. Patients suffering from shortness of breath and anxiety are often unable to separate psychogenic and pulmonary origins of dyspnea. Also cardiac diseases, especially heart failure, cause dyspnea. These might also be reasons behind increased sensation of dyspnea. In our study the percent of patients with a psychiatric condition that requires medication was 19% in physically active and 23% in inactive patients. Smoking was slightly more common among inactive patients and had weak independent association to inactivity. Smoking and sensation of dyspnea are also connected, and it is hard to estimate how large a portion of dyspnea comes from smoking. It is also known that people with depression or anxiety are more often smokers than the general population. Psychiatric diseases, smoking and dyspnea seem to form an intertwined net where causalities are hard to comment on.

Lung-specific HRQoL was significantly better among physically active patients, but interestingly this did not reflect on the general HRQoL, which did not differ between active and inactive patients. General HRQoL takes a wide variety of aspects into account. For example, comorbidities can affect it remarkably. Because active and

inactive patients were very similar in several aspects, such as age and gender distribution, smoking status and profile of comorbidities, it can be postulated that these similarities are displayed in general HRQoL results.

6.3. Smoking cessation

The smoking cessation patterns, success and challenges among COPD patients are hard to study because of the high rate of smoking cessation when receiving the diagnosis. Some people manage to quit smoking even before they are diagnosed with COPD. This kind of early quitting was more common among men in our cohort. CVD was more common among these early quitters, and it is possible that men had better quitting rates because they had a higher incidence of CVD that motivated them to quit smoking even before receiving the COPD diagnosis. Receiving a COPD diagnosis seems to be a strong wake-up call for many patients. In our study, 31% of patients had quit smoking after the diagnosis but before entering the study. Patients who had quit smoking were older, had more pack years and comorbidities and lower lung functions, but importantly, less alcohol abuse or psychiatric conditions, than non-quitters. More advanced disease, which is likely to be more symptomatic, seems to explain smoking cessation. These findings are in accordance with a large study among US veterans. (Adams, Pugh et al. 2006) Older age and accumulating diseases seem to increase the motivation to quit, and at the same time these patients have adequate mental resources to tackle their addiction. The higher mortality among non-quitters, even though they are younger and have better lung functions, is very likely to result from a higher prevalence of multiple self-harming behaviors (smoking, alcohol abuse) and psychiatric conditions.

6.4. Effects and possible mechanisms of CHRNA3/5 locus on COPD related variables

Nicotinic acetylcholine gene cluster CHRNA3/5 has been shown to associate with lung functions, COPD, lung cancer, coronary disease, addiction behavior and personality related features in other data sets. (Thorgeirsson, Geller et al. 2008, Pillai, Ge et al. 2009, Mohamed Hoesein, Wauters et al. 2013, Wang, Grucza et al. 2009) Results from these studies are in accordance with our findings, as we presented an association between rs1051730 SNP and heavy smoking and cancer prevalence. A novel finding in our study, however, was the SNP's association directly to mortality that had not been reported earlier. Even though the study cohorts were small, the association to mortality was found in two independent cohorts (CAD and male smoker cohort), which emphasizes the importance of the finding.

The mechanisms through which nicotinic acetylcholine gene affects aforementioned variables are currently unclear. One theory is that CHRNA3/5 plays a part in the

formation of nicotine addiction, which would result in heavy smoking and thus in development of lung diseases. (Budulac, Vonk et al. 2012) In some studies, however, the association between the gene cluster and lung cancer has been found also in never-smokers, indicating that the effect doesn't result only from heavy smoking. (VanderWeele, Asomaning et al. 2012) The results of our study seem to point to this direction too. In multivariate Cox regression SNP had independent explanatory value on mortality, although smoking variables had been taken into analysis. CHRNA3/5 locus has been reported to associate with not only lung-related outcomes but also addictions (nicotine dependence, alcohol abuse), psychiatric conditions and personality-related phenotypes. (Wang, Grucza et al. 2009, Hong, Yang et al. 2011, Winterer, Mittelstrass et al. 2010) Here the mechanism has been assumed to be direct: through nicotine mediated pathways in central nervous system. It is possible that the increased risk of mortality is mediated through both direct and indirect effects of CHRNA3/5 polymorphism.

Large GWAS studies are an effective technique for finding new common (MAF >5%) genetic markers. But to evaluate how a genetic marker affects a patient's life and what kind of effect it has on symptoms, drug response or survival, testing and validation of the marker in smaller, well-characterized, often longitudinal study populations is needed. CAD cohort is a very good data set for this purpose because we have comprehensive data on patients' characteristics over a long period of time. Careful data collection and characterization of study population is important, so that the effects of other phenotypic and clinical features apart from genetic markers can be analyzed and the effect of the genetic marker in real life setting can be estimated. Only after this kind of analysis should genetic testing be considered.

6.5. Chronic airway diseases in research

COPD as a disease presents challenges for research. First, it is a heterogenic disease with slow progression that usually takes decades. Follow-up studies are needed to find out causalities in morbidity and mortality. Due to the nature of the disease and its risk factors (long tobacco exposure), the follow-up times should be several years to the least, which is both expensive and time-consuming. Some large prospective follow-up studies have been made, for example ECLIPSE study (Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points) but even in that study the follow-up time was only 3 years. (Agusti, Calverley et al. 2010) Large randomized controlled trials, e.g. TORCH, UPLIFT and TIOSPIR, have focused on optimizing treatment in COPD and have had follow-ups of 2 to 4 years. (Calverley, Anderson et al. 2007, Tashkin, Celli et al. 2008, Wise, Anzueto et al. 2013)

Cross-sectional studies are easier and faster to perform than longitudinal studies, but although they provide a lot of important information on COPD, they give information about associations and do not show causalities. Retrospective data is a possibility to get longitudinal data without long follow-up periods but is prone to recall bias, especially when it comes to COPD risk factors like smoking.

Comorbidities are another aspect that often complicates COPD studies. Several common comorbidities have similar symptoms to COPD. Dyspnea can be caused by pulmonary or cardiac diseases or psychiatric conditions like anxiety. In COPEX study, the number of patients suffering from moderate to severe dyspnea was 50% (MMRC score 2–4). Generic and lung-specific HRQoL, exercise activity and mobility score had stronger correlations to dyspnea than lung functions, demonstrating the complexity of dyspnea etiology. This finding is in line with earlier results showing that dyspnea correlates better with 6 minutes walking distance test than with FEV1. (Spruit, Watkins et al. 2010) Dyspnea is, however, an important symptom of COPD, and COPD can cause poor oxygenation that is one of the factors leading to low level of physical activity. This, in turn, can promote the development of several comorbidities like CVD. The low-grade inflammation that is present in COPD can be one of the causative agents of other chronic conditions. (Barnes, Celli 2009) At the same time comorbidities can e.g. promote physical inactivity that accelerates COPD progression. Because of these confounding factors, COPD patients with several comorbidities are often excluded from randomized clinical trials. This makes the interpretation of the trials easier, but at the same time it is questionable whether the results reflect the reality of COPD patients. In our cohort, all patients regardless of comorbid profile are included, which may give us a more realistic picture of the disease.

6.6. Strengths and challenges of the study design

A study design that combines prospective and retrospective data opens many possibilities. A large amount of data can be extracted fast and with relatively low cost from medical records to characterize the patients. If it becomes clear that some interesting data cannot be acquired from retrospective data, it can always be collected during the follow-up. Prospective data gives important information on patients' well-being during the study. Mortality data can be collected during the follow-up and the risk factors analyzed. Prospective study can react fast if new, interesting study questions requiring new data arise. In our study such data was e.g. MMRC scores and FTND questionnaire. Repeated gathering of prospective data (frequent follow-ups) give an opportunity to analyze changes and trends in collected variables.

In our study we collected the retrospective data from medical records that are gathered for treatment, not for research. There are both positive and negative aspects when this kind of data is used in research. On the positive side, there is not much recall bias because all diagnoses, measurements and treatments are documented in the medical records directly after they are administered. However, there might be differences in treatments depending on the year, hospital and even individual doctor. Diagnostic criteria also change over years as we can see in COPD where diagnostic criteria are based on GOLD recommendations and old Finnish criteria are no longer in use. Although medical records data is not very sensitive to recall bias, it is important to keep in mind that variables such as starting and quitting smoking and smoking

intensity are always patient-reported data, regardless of whether it is collected from medical records or asked directly from patients. The chances are, however, that if a patient has e.g. quit smoking during the time period of which we have medical records, we can pin the quitting time down more accurately than when asking it from the patient. Medical records data includes all data on the patients, not only the variables researchers find interesting when planning a study. There is also a large amount of data that could not be accessed any other way; it is highly unlikely that patients would remember e.g. their lung function measurements five years after a spirometry test. Medical records are a source of large amount of data that might not have been planned to be used initially but that can provide interesting new data and possibilities later on.

One of the negative aspects of medical records data is that not all data is found from all patients. This can lead to exclusion of otherwise eligible study subjects or to smaller data groups in statistical analyses, which, in turn, reduces the statistical power of the study. When data is collected from medical records, there are no common time points when patients have e.g. received a diagnosis or had a spirometry test. This complicates the build-up of data sets in studies where time is included in the analyses. Moreover, although the data extraction from existing medical records is faster and cheaper than with prospective studies, it can still take a lot of time and require a specialist to evaluate the medical texts and their context.

Prospective studies are time consuming and thus expensive to carry out. They have, however, fewer sources of bias than retrospective studies, enable collection of data that could not be acquired by retrospective studies and offer a better possibility to draw conclusions about causality. To get comprehensive data with a prospective study, the number of patients that drop out from the study needs to be as low as possible. In our study, the drop-out rates were very low, probably partly due to the active work of our research nurses who kept in touch and provided information to the patients, and partly because of regular follow-up questionnaires that reminded patients about the study and gave information of the proceeding of the study.

A challenge with a study that combines both retrospective and prospective data is that it takes a long time to get the final, complete data set. During the prospective part of the study, several different data sets and subcohorts can be constructed for different study questions, and retrospective variables can be collected for only a subcohort. Different study questions may require exclusion of certain patients or data, so both the number of patients and variables in different subcohorts and analyses may vary. When all prospective data is collected, creating one conclusive data set with all variables used in earlier studies can present a challenge.

CAD study cohort recruitment was done among patients who were treated in special health care. Thus the results from this cohort may not be generalizable to all asthma, COPD or ACOS patients. On the other hand, this cohort is focused on patients with severe symptoms and exacerbations (most common reasons for visit specialist health care) and establishes factors that affect the disease and prognosis of patients who have required specialist care.

7. CONCLUSIONS

In this study we describe a real life chronic obstructive airways disease cohort. All eligible patients were included in the study without further selection or exclusions. The patients were recruited from four hospital in two hospital districts, and this is the largest Finnish cohort of chronic airway disease patients with such a severe disease.

Epidemiology of asthma and COPD overlap syndrome has not been studied in Finland before. An important finding was that ACOS patients suffer from significantly poorer health related quality of life than either asthma or COPD patients. Our results suggest that extra care should be paid not only to ACOS patients' medication, but also to other aspects of patients' life, promoting their well-being, quality of life and management of disease.

Physical activity and exercise habits among the Finnish COPD patients were determined for the first time. In our cohort approximately half of COPD patients were physically inactive. However, levels of physical activity varied greatly among the study subjects. Physical activity was not strongly affected by the level of obstruction or comorbidities. Subjective sensation of dyspnea was a very strong marker of physical inactivity and poor HRQoL. More emphasis should be focused on encouraging patients with a strong sensation of dyspnea to exercise.

As a conclusion, it seems that a large part of COPD patients' activity cannot be explained with the severity of disease, comorbidities or other characteristics. Motivation and attitude seem to be important factors as well as controlling the sensation of dyspnea. This presents challenges to health care but also gives a promising message that even patients with severe COPD can and should exercise and be physically active.

Although smoking is generally considered very addictive, and the majority of COPD patients have long smoking histories, more than half of CAD cohorts' COPD patients had managed to quit smoking before entering the study. This is a very interesting and promising result and shows that encouragement and interventions for smoking cessation are important in COPD patients. When analysing the comorbidity profile of COPD patients we found that cardiovascular diseases, as well as older age and larger number of pack years associated to succeeded smoking cessation. Psychiatric conditions, alcohol abuse and failing at smoking cessation clustered on a subgroup of patients. They also had higher mortality than those patients who had managed to quit smoking. It is hard to comment on causality between COPD, addictive behavior, like alcohol abuse and smoking, and psychiatric symptoms such as anxiety and depression. It is clear, however, that this patient groups needs special attention and help in smoking cessation.

Nicotinic acetylcholine gene cluster CHRNA3/5 SNP rs1051730 was associated to all-cause mortality in two independent Finnish cohorts with study subjects of long smoking history. The association between rs1051730 and mortality has not been

reported earlier. In CAD cohort the rs1051730 was also associated to risk of any type of cancer and in cohort of male smokers, to heavy smoking. In order to validate the genetic markers, detailed and well-defined data sets with long follow-up times are needed. Retrospective CAD cohort is a good data set for this purpose because we have comprehensive data on patients' characteristics from a long period of time.

CAD study has shown us that COPD patients are not a homogenous group of patients, but subgroups of patients with a different spectrum of comorbidities, level of physical activity, genetic susceptibility and thus different prognosis. These subgroups need different approaches on treatment and interventions. Personalized health care and medicine that focus on the treatment needs of individual patients could improve survival and HRQoL and thus provide better long-term treatment and prognosis.

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