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Smoking patterns and lung function:

A longitudinal twin study

Maria Hukkinen, MD

Department of Public Health

Hjelt Institute

University of Helsinki

Finland

ACADEMIC DISSERTATION

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Supervised by

Professor Jaakko Kaprio

Department of Public Health, Hjelt Institute and Institute for Molecular Medicine,

University of Helsinki

National Institute for Health and Welfare, Helsinki, Finland

and

Docent Tellervo Korhonen

Department of Public Health, Hjelt Institute, University of Helsinki

National Institute for Health and Welfare, Helsinki, Finland

Reviewed by

Professor Vuokko Kinnula

University of Helsinki

Pulmonary division, Department of Medicine,

Helsinki University Central Hospital, Helsinki, Finland

and

Dr. Philip Tønnesen

Department of Pulmonary Medicine

Gentofte University Hospital

Copenhagen, Denmark

Opponent

Docent, Research Director Riitta Luoto

UKK Institute for Health promotion research, Tampere, Finland

National Institute for Health and Welfare, Helsinki, Finland

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Abstract

In many Western countries, the average cigarette consumption among smokers has decreased, with occasional smoking and daily light smoking (<1-4 cigarettes per day, CPD) becoming more common. Despite this decrease in smoking frequency, the prevalence of chronic obstructive pulmonary disease (COPD), a disorder characterized by progressive decline in lung function, continues to rise globally. The risk for COPD increases with smoking exposure; however, not all smokers develop COPD. Genetic factors partly explain the differences in lung function between individuals, and the susceptibility of some smokers to COPD. The proportion of genetic effects influencing lung function varies across time and populations. No earlier research on the genetic and environmental determinants of lung function or on the phenomenon of light smoking exists in the Finnish population. Further, the association between low-rate smoking patterns and COPD remains partly unknown. This thesis aimed to study the prevalence and consistency of light smoking longitudinally in the Finnish population, to assess the characteristics of light smokers, and to examine the risks of chronic bronchitis and COPD associated with changing smoking patterns over time. A further aim was to estimate longitudinally the proportions of genetic and environmental factors that explain the inter-individual variances in lung function.

Data from the Older Finnish Twin Cohort, including same-sex twin pairs born in Finland before 1958, were used in this study. Three surveys were conducted in 1975, 1981, and 1990, including accurate and consistent definitions of smoking and chronic bronchitis symptoms. Data used in Studies I, II, and IV were mainly derived from the 1975 and 1981 surveys. A subsample of the cohort, female twins born in 1924-1937 who attended spirometry in 2000 and 2003, formed the sample of Study III. The genetic and environmental influences on lung function were estimated by using genetic modeling on the repeated spirometry measures. In Study IV, a record-linkage study was performed by combining the twin cohort data with that obtained from the Finland's Social Insurance Institution (SII). The SII's data on reimbursement eligibilities and medication purchases were used to define COPD.

This thesis reveals that light smoking is associated with female sex, a healthier lifestyle, and a better education. Between 1975 and 1990, the proportion of light smokers in Finnish adult smokers remained constant at 8%. However, on an individual level, this pattern was rarely constant. Light smoking, reducing from heavier smoking to light smoking, or relapsing to light smoking after quitting are among patterns associated with an increased risk

of chronic bronchitis and COPD. Constant light smoking is associated with an increased use of inhaled anticholinergics, a medication for COPD. In addition to smoking, other environmental factors substantially influence lung function in the elderly. During a three-year follow-up, new environmental effects influencing spirometry values emerged, whereas the genes affecting lung function remained mostly the same.

In conclusion, no safe level of daily smoking exists with regard to pulmonary diseases. Even daily light smoking in middle-age is associated with increased respiratory morbidity. Light smoking is rarely a constant pattern, and light smokers need cessation interventions in order to prevent them from progressing towards heavier smoking. Smoking reduction does not decrease the risk of COPD and should not be recommended as an alternative to quitting smoking. In elderly people, attention should also be drawn to other factors that can prevent poor lung function**.**

Tiivistelmä

Useissa länsimaissa tupakoitsijoiden päivittäinen savukkeiden määrä on pienentynyt ja satunnaistupakointi sekä määrältään vähäinen päivittäistupakointi (1-4 savuketta päivässä) ovat yleistyneet. Tupakointimäärien vähenemisestä huolimatta keuhkoahtaumataudin esiintyvyys maailmassa jatkaa kasvuaan. Keuhkoahtaumatauti on etenevä sairaus, jossa keuhkojen toiminta on pysyvästi heikentynyt. Vaikka altistus tupakansavulle onkin tärkein keuhkoahtaumataudin riskitekijä, kaikki tupakoitsijat eivät sairastu. Geneettiset tekijät vaikuttavat sairastumisalttiuteen ja selittävät osan yksilöiden välisistä eroista keuhkojen toimintakapasiteetissa. Geneettisten tekijöiden selitysosuus keuhkotoiminnan yksilöiden välisestä vaihtelusta riippuu tutkimusajankohdasta ja –väestöstä. Suomalaisessa väestössä ei ole aiemmin tutkittu määrältään vähäistä tupakointia eikä geneettisten tekijöiden ja ympäristötekijöiden vaikutusta keuhkotoimintaan. Erilaisten vähäisten tupakointitapojen ja niissä tapahtuvien muutosten yhteys krooniseen bronkiittiin ja keuhkoahtaumatautiin tunnetaan myös puutteellisesti. Tämän väitöskirjan tavoitteena oli tutkia pitkittäisasetelmalla suomalaisessa väestössä määrältään vähäisen tupakoinnin esiintyvyyttä ja pysyvyyttä, 1-4 savuketta päivässä tupakoivien ominaispiirteitä, sekä erilaisten vaihtelevien ja muuttuvien tupakointitapojen yhteyttä krooniseen bronkiittiin ja keuhkoahtaumatautiin. Lisäksi tavoitteena oli arvioida geneettisten tekijöiden ja ympäristötekijöiden selitysosuuksia yksilöiden välisestä keuhkotoiminnan vaihtelusta.

Aineistona käytettiin Suomen kaksoskohorttitutkimuksen kolmea kyselyaineistoa vuosilta 1975, 1981 ja 1990. Kohorttiin oli poimittu väestörekisteristä Suomessa ennen vuotta 1958 syntyneet kaksoset. Kyselyissä on tutkittu tupakointitavat sekä kroonisen bronkiitin oireet yksityiskohtaisesti ja yhtenevällä tavalla. Ensimmäisen, toisen ja neljännen osatyön aineiston muodostivat lähinnä vuosien 1975 ja 1981 kyselyihin vastanneet henkilöt. Kolmannen osatyön aineistona käytettiin kaksoskohortin alaotosta, vuosina 1924-37 syntyneitä naiskaksospareja, jotka osallistuivat spirometriamittauksiin vuosina 2000 ja 2003. Geneettisten tekijöiden ja ympäristötekijöiden selitysosuudet spirometriatulosten yksilöiden välisestä vaihtelusta arvioitiin käyttämällä geneettistä mallinnusta. Neljännessä osatyössä kaksoskohorttiaineistoon yhdistettiin Kansaneläkelaitoksen rekisteriaineistoa, josta saatua tietoa lääkeostoista sekä lääkkeiden erityiskorvattavuuksista käytettiin keuhkoahtaumataudin määrittelemiseksi.

Tutkimuksessa havaittiin, että määrältään vähäinen tupakointi oli yksilötasolla harvoin pysyvä tapa, vaikka tupakoitsijoiden joukossa 1-4 savuketta päivässä tupakoivien osuus oli 8% kaikissa mittauspisteissä. Kun 1-4 savuketta päivässä tupakoivia verrattiin yli 20 savuketta päivässä tupakoiviin, oli vähäinen tupakointi todennäköisempää naisilla, terveellisiä elintapoja noudattavilla ja hyvin koulutetuilla ihmisillä. Runsaampien tupakointimäärien lisäksi vähäinen päivittäistupakointi, siihen vähentäminen runsaamman tupakoinnin jälkeen tai tupakoinnin uudelleen aloittaminen lopettamisen jälkeen olivat yhteydessä suurentuneeseen kroonisen bronkiitin ja keuhkoahtaumataudin riskiin. Pysyvä 1-4 savukkeen päivittäistupakointi oli yhteydessä lisääntyneeseen inhaloitavien antikolinergien käyttöön, joka on eräs keuhkoahtaumataudin lääkehoidoista. Tupakoinnin lisäksi myös muut ympäristötekijät vaikuttavat huomattavasti keuhkotoimintaan vanhemmalla iällä. Kolmen vuoden seurannan aikana havaittiin, että spirometria-arvoihin vaikuttavat geneettiset tekijät pysyivät valtaosin samoina, kun taas ympäristötekijöistä valtaosa oli kullekin seurantapisteelle ominaisia.

Tutkimustulokset tukevat käsitystä siitä, että vähäinenkin tupakointi voi vahingoittaa keuhkoja. Vähäinen päivittäistupakointi keski-iässä lisää myöhemmin kehittyvien hengitysteiden sairauksien riskiä. Määrältään vähäinen tupakointi on harvoin pysyvä tapa. Jotta 1-4 savuketta päivässä tupakoivat eivät siirtyisi runsaampaan tupakointiin, he tarvitsevat tukea tupakoinnin lopettamiseen. Tupakoinnin vähentämistä ei voi suositella vaihtoehtona lopettamiselle, sillä tupakoinnin vähentäminen ei pienennä keuhkoahtaumataudin kehittymisen riskiä. Jotta voitaisiin ehkäistä ikääntyvien ihmisten keuhkotoiminnan ennenaikaista alenemista, tulee kiinnittää huomiota myös muihin riskitekijöihin kuin tupakointiin.

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List of original publications

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals (I-IV):

I. Hukkinen M., Kaprio J., Broms U., Koskenvuo M., Korhonen T. Characteristics and consistency of daily light smokers: Longitudinal study among Finnish adults. *Nicotine and Tobacco Research*, 2009 Jul;11(7):797-805.

II. Hukkinen M., Korhonen T., Broms U., Koskenvuo M., Kaprio J. Long-term smoking patterns predicting self-reported chronic bronchitis. *Journal of COPD*, 2009 Aug;6(4):242-9.

III. Hukkinen M., Kaprio J. Broms U., Viljanen A., Kotz D., Rantanen T., Korhonen T. Heritability of lung function: A twin study among never-smoking elderly women. *Twin Research and Human Genetics,* 2011 Oct;14(5):401-407.

IV. Hukkinen M., Korhonen T., Heikkilä K., Kaprio J. Association between smoking behavior patterns and chronic obstructive pulmonary disease: A long-term follow-up study among Finnish adults. *Annals of Medicine,* published online 25.5.2011.

Abbreviations

A = additive genetic effects

AAT = alpha$_1$ antitrypsin

AIC = Akaike's information criterion

ATS = American Thoracic Society

BMI = body mass index

BTS = British Thoracic Society

C = shared environmental effects

CI = confidence interval

COPD = chronic obstructive pulmonary disease

CPD = cigarettes per day

CRP = C-reactive protein

CYP2A6, CYP2B6 = cytochrome P450 enzymes, responsible for nicotine metabolism

D = dominant genetic effects

DZ = dizygotic

E = nonshared environmental effects

ECRHS = European Community Respiratory Health Survey

ERS = European Respiratory Society

FEV1 = forced expiratory volume in one second

FinEsS = Finnish, Estonian, and Swedish respiratory survey

FITSA = Finnish Twin Study on Aging

FVC = forced vital capacity

G x E interaction = gene-environment interaction

GE correlation = gene-environment correlation

GOLD = Global Initiative for Obstructive Lung Disease

GWA = genome-wide association

HHIP = hedgehog interacting protein

HR = hazard ratio

ICC = intra-class correlation coefficient

LLN = lower limit of normal

MMP12 = matrix metalloproteinase

MRC = British Medical Research Council

MZ = monozygotic

nAChR = nicotinic acetylcholine receptor

NIV = noninvasive ventilation

OR = odds ratio

SD = standard deviation

SEM = structural equation modeling

SNP = single-nucleotide polymorphism

TLC = total lung capacity

VC = vital capacity

WHO = World Health Organization

XZ = undetermined zygosity

1. Introduction

Smoking is the most important preventable cause of premature morbidity and mortality (Ezzati et al. 2002). Among leading causes of deaths from smoking are cardiovascular diseases, chronic obstructive pulmonary disease (COPD), and lung cancer (Ezzati & Lopez 2003). The total number of tobacco-attributable deaths, 5.4 million in 2005, has been estimated to reach 8.3 million by 2030 and to account for 10% of all deaths globally. COPD-related mortality is increasing sharply, particularly in developing countries, where the number of all smoking-induced deaths has been projected to double by 2030. In high-income countries, COPD is the major chronic disease for which death rates are increasing (World Health Organization 2004, Mathers & Loncar 2006, Global Alliance against Chronic Respiratory Diseases 2007).

Awareness of the deleterious health effects of smoking started to spread in the 1950s. Large epidemiological studies were designed when the increase in lung cancer rates was observed, and soon results on the associations between smoking and cardiovascular diseases, chronic bronchitis, and emphysema were also published (U.S. Surgeon General's Advisory Committee on Smoking and Health 1964, Peto 1994). Chronic bronchitis and emphysema had been considered as different entities for decades, but were recognized to be clinical manifestations of the same disease in the 1960s. The common term chronic obstructive pulmonary disease was first introduced in 1965 (Fishman 2005, Petty 2006). In 1977, Fletcher described the association between smoking and accelerated lung function decline (Fletcher & Peto 1977).

In Finland, the prevalence of smoking among men was almost 70% in the 1950s, decreasing to 45% by 1965-1970. In contrast, only about 13% of women smoked at the beginning of the 1960s, whereas the proportion had increased to 17% by 1978 (Helakorpi et al. 2004). The Tobacco Act, passed in 1976, restricted tobacco advertising and smoking in public places, and set the age limit of tobacco purchases to 16 years (Helakorpi et al. 2008a). Since then, the prevalence of smoking in men has steadily decreased. In women, by contrast, smoking rates have been declining only since the 1990s. In the 21st century, amendments have been made to the Tobacco Act, and the trend of decreasing smoking rates continues: the proportion of former smokers has increased, and smokers' daily number of cigarettes has decreased (Helakorpi et al. 1999, Helakorpi, Laitalainen & Uutela 2010). However, light smoking (1-4 cigarettes per day), an increasingly common pattern globally (Okuyemi et al.

2002, Zhu et al. 2003, Pierce, White & Messer 2009), has not been studied in the Finnish population.

Fletcher noted that some smokers had a steeper decline in lung function than others, and termed them 'susceptible smokers' (Fletcher & Peto 1977). Genetic factors have been stated to account for the observed differences between subjects in susceptibility to cigarette smoke and COPD development (Mannino & Buist 2007, Sorheim et al. 2010). Heritability refers to the proportion of inter-individual variance explained by genetic factors. Reported heritability estimates for lung function have been inconsistent, varying across populations. Smoking is one factor complicating these heritability studies, as it modifies the expression of genes influencing lung function (Silverman et al. 1998, Wilk et al. 2000).

In many populations, different low-rate smoking patterns are increasing (Okuyemi et al. 2002, Zhu et al. 2003, Pierce, White & Messer 2009). Previous smoking research has focused mainly on heavier smoking patterns, and the health effects of low-rate smoking remain partly unknown. Research evidence has shown that light smoking also causes cardiovascular diseases and lung cancer as well as increased mortality (Bjartveit & Tverdal 2005, Schane, Ling & Glantz 2010). Light and passive smoking seem to be associated also with respiratory symptoms (Radon et al. 2002, Amigo et al. 2006); however, whether light smokers are at risk for COPD remains to be elucidated. Moreover, longitudinal studies on the respiratory consequences of reduced smoking are few, although the average daily amount of cigarettes among smokers has diminished considerably.

The Older Finnish Twin Cohort includes comprehensive data on smoking patterns and respiratory symptoms of over 30,000 Finnish twins over a 15-year period. Spirometry has been performed for a subgroup of this cohort. The cohort offers a unique opportunity to study the changes that take place in smoking over time, reflecting the smoking patterns in the population. Moreover, by combining Finland's Social Insurance Institution’s high-coverage medication data with the twin cohort data, the development of respiratory diseases can be traced even decades after the end of the cohort. This thesis focused on investigating the subgroup of light smokers, the heritability of lung function, and the associations of different smoking patterns with chronic bronchitis and COPD by taking full advantage of these extensive databases.

2. Review of the literature

2.1. Definitions of different smoking patterns

Traditionally, researchers have grouped smokers into occasional (intermittent, nondaily), current daily, and former smokers. A person reporting having consumed at least 100 cigarettes during his/her lifetime is usually considered an ever smoker, although other definitions are also sometimes used (Giovino 2002, Helakorpi, Laitalainen & Uutela 2010). Based on the frequency of cigarette consumption, current smokers are divided into daily and occasional smokers. Those ever smokers who have not smoked for a certain time period, often for 6 or 12 months, are considered former smokers (Hughes et al. 2003). In epidemiological studies, however, it is common to classify all ever smokers who state that they no longer smoke as former smokers (Giovino 2002).

Daily smokers have usually been subdivided into those consuming <15, 15-24, and over 25 cigarettes per day (CPD) (Okuyemi et al. 2002, Helakorpi, Prättälä & Uurtela 2008), those with greater consumption being the main focus of smoking studies (Shiffman 2009). In the 1990s, researchers became interested also in daily smokers consuming <5 and <10 CPD (Shiffman 1989, Rosengren, Wilhelmsen & Wedel 1992, Shiffman et al. 1994, Hajek, West & Wilson 1995, Owen et al. 1995). Such smokers have been called, for example, 'chippers' (Kassel et al. 1994, Shiffman et al. 1994), 'low-rate smokers' (Owen et al. 1995, Zhu et al. 2003), 'low-level smokers' (Hyland et al. 2005), 'light smokers' (Rosengren, Wilhelmsen & Wedel 1992, Bjartveit & Tverdal 2005), and 'very light smokers' (Levy, Biener & Rigotti 2009). The term 'light smoking' has been used to describe smokers consuming up to 20 CPD, although usually 10 CPD (Husten 2009, Levy, Biener & Rigotti 2009) or 5 CPD (Rosengren, Wilhelmsen & Wedel 1992, Bjartveit & Tverdal 2005) are used as cut-off levels. Light and intermittent smokers are sometimes also grouped together in a category called 'LITS' (Pierce, White & Messer 2009, Shiffman 2009).

2.2. Low-rate smoking patterns

2.2.1. Prevalence of low-rate smoking patterns

While the prevalence of smoking is decreasing in Western countries, the proportions of light and occasional smokers are on the rise (Okuyemi et al. 2002, Zhu et al. 2003, Giskes et al. 2005, Pierce, White & Messer 2009). Smoking cessation rates have increased, and daily

smokers' average number of cigarettes has decreased in recent decades (Pierce, White & Messer 2009, Helakorpi, Laitalainen & Uutela 2010, Marques-Vidal et al. 2011). The observed trend is most evident among well-educated subjects (Giskes et al. 2005). In Finland, the proportion of smokers consuming 1-14 CPD increased between 1999 and 2009, while consumption of more CPD became less prevalent. Similarly, the proportion of daily smokers decreased from 23.3% to 18.6%, and the proportion of quitters increased from 17.1% to 20.3% (Helakorpi et al. 1999, Helakorpi, Laitalainen & Uutela 2010). In Finland, the prevalence of occasional smoking in the population between 1978 and 2009 has remained constant at 6-6.5% (Helakorpi et al. 1999, Luoto, Uutela & Puska 2000, Helakorpi, Laitalainen & Uutela 2010). Thus, as daily smoking has become less prevalent, the relative proportion of occasional smokers has increased. Occasional smoking is particularly common among young adults and women (Luoto, Uutela & Puska 2000, Korhonen et al. 2009).

International studies in the 1990s reported that the proportion of smokers consuming <5 CPD is 4-8% among daily smokers (Rosengren, Wilhelmsen & Wedel 1992, Owen et al. 1995, Zhu et al. 2003). In the US, the proportion of light and occasional smokers among all smokers has recently been estimated to be 20-30% (Shiffman 2009), and the increase has been greatest among young adults (Pierce, White & Messer 2009). Light smoking is particularly common among women, adolescents, and well-educated persons (Owen et al. 1995, Okuyemi et al. 2002, Hyland et al. 2005, Levy, Biener & Rigotti 2009, Pierce, White & Messer 2009). No studies on light smokers in the Finnish population have been published.

Light smokers form a heterogeneous subgroup of daily smokers, where some are well established in this pattern, while others are progressing towards heavier use or towards cessation (Owen et al. 1995, Okuyemi et al. 2002, Zhu et al. 2003, Hyland et al. 2005, Levy, Biener & Rigotti 2009). Longitudinal studies report that light smokers are rarely consistent in their smoking habit (Zhu et al. 2003, Etter 2004, Levy, Biener & Rigotti 2009), although some individuals can maintain this pattern for long periods of time (Owen et al. 1995, Hyland et al. 2005).

2.2.2. Factors contributing to low-rate smoking

Light smokers have been suggested to be more motivated by social and enjoyment factors than by nicotine addiction (Shiffman et al. 1994). Some studies have found that light smokers do not suffer from withdrawal symptoms while abstinent (Shiffman 1989, Okuyemi et al. 2002). Indeed, it has been suggested that by smoking 1-4 CPD a steady-state nicotine level

cannot be maintained (Shiffman et al. 1990). However, many smokers accustomed to heavier smoking rates are able to reduce their smoking for substantial periods of time (Hughes 2000).

Smoking restrictions at home and at the workplace as well as society's tobacco prohibitions and high taxation of tobacco products are associated with reduced smoking (Okuyemi et al. 2002, Jha et al. 2006, Pierce, White & Messer 2009). Also in Finland, changes in tobacco policy have contributed to the decline in daily smoking (Helakorpi et al. 2004, Helakorpi 2008). The Tobacco Act was passed in 1976, and amendments in 1995 and 2007 raised the age limit of purchasing tobacco products and prohibited smoking in many public places (Helakorpi et al. 2004, Helakorpi et al. 2008b). Further amendments in 2010 aim at ending the use of tobacco products in Finland. Prevalence of smoking in men has been decreasing since the 1970s, whereas smoking rates in women increased slightly until the mid-1980s, remained at a constant level for a while, and have been declining since the 1990s (Helakorpi, Laitalainen & Uutela 2010). Attitudes towards smoking cessation restrictions are positive in Finland (Nieminen et al. 2010).

Some smokers, e.g. pregnant women, are worried about the health effects of smoking and aim at 'harm reduction' by smoking fewer cigarettes (Okuyemi et al. 2002). Light smokers have been reported to be less impulsive and more self-disciplined than heavier smokers (Shiffman 1989, Kassel et al. 1994). Compared with heavier smokers, light smokers attempt quitting more often (Owen et al. 1995, Zhu et al. 2003, Hyland et al. 2005) and are better educated (Hajek, West & Wilson 1995, Pierce, White & Messer 2009). Finnish studies have found that occasional smokers are better educated, physically more active, and have a healthier diet than regular smokers (Luoto, Uutela & Puska 2000, Korhonen et al. 2009), suggesting that health-related issues might restrict their smoking patterns. Whether Finnish light smokers exhibit similar characteristics is thus far unknown.

Use of snus, a moist oral tobacco powder, is common in Sweden, whereas only 3% of the population in neighboring Finland report using it (Patja et al. 2009). In Sweden and Norway, substantial proportions of snus users concomitantly smoke cigarettes (Patja et al. 2009, Lund et al. 2011). This type of dual use may help smokers to adapt to small amounts of daily cigarettes. However, such behavior was unlikely to occur in Finland in the 1970s-1980s, when the use of snus was even more rare than today (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2007).

Genetics influence the age of smoking initiation, the daily amount smoked, and nicotine dependence (Broms et al. 2006, Ho & Tyndale 2007, Thorgeirsson et al. 2010).

Reported heritability estimates for nicotine dependence and smoking behavior have been consistently high, albeit varying (Broms et al. 2006, Ho & Tyndale 2007, Korhonen et al. 2009). Multiple genome-wide association studies (GWAs) have demonstrated that the nicotinic acetylcholine receptor (nAChR) gene complex on chromosome 15 is associated with smoking behavior and nicotine dependence, as well with lung cancer and COPD (Saccone et al. 2010, Siedlinski et al. 2011). However, genetic variants at this locus explain only 1% of the observed variance in the amount smoked (Thorgeirsson et al. 2008). Another example of the genetic variance affecting smoking behavior is the nicotine-metabolizing enzyme CYP2A6 (Thorgeirsson et al. 2010, Siedlinski et al. 2011). Individuals with a slow variant of CYP2A6 experience less craving and withdrawal symptoms because of the slower inactivation of nicotine (Okuyemi et al. 2002, Ho & Tyndale 2007). This variant may be one reason why some smokers can maintain low consumption levels. However, smoking reducers sometimes compensate for nicotine reduction by inhaling more deeply (Scherer 1999). Genomic regions encoding for example other nicotinic receptors and CYP2B6 have also been reported to be associated with smoking behavior (Thorgeirsson et al. 2010), and smoking has been shown to modify genetic expression of multiple traits (Zeller et al. 2010). Nicotine dependence and smoking are, however, complex disorders, where the phenotype is likely to be a result of an interaction between environmental factors and multiple genetic factors (Ho & Tyndale 2007). Accordingly, genetics cannot totally explain the phenomenon of light smoking, particularly its increasing prevalence.

2.2.3. Health effects

Light smoking has been demonstrated to be associated with an almost similar risk for cardiovascular diseases as heavier smoking patterns (Schane, Ling & Glantz 2010). Light smoking increases cardiovascular and overall mortality (Luoto, Uutela & Puska 2000, Bjartveit & Tverdal 2005), as well as the risk for lung and gastrointestinal cancers (Rosengren, Wilhelmsen & Wedel 1992, Schane, Ling & Glantz 2010). Smoking 1-4 CPD has also been shown to impair reproductive health (Schane, Ling & Glantz 2010).

No consensus exists about whether smokers can diminish their health risks by reducing the number of daily cigarettes (Hughes & Carpenter 2006). Cohort studies report that smoking reduction has no effect on mortality (Godtfredsen et al. 2002, Tverdal & Bjartveit 2006), hospital admissions due to COPD (Godtfredsen et al. 2002), or risk of myocardial infarction (Godtfredsen et al. 2003). However, large reductions in smoking have

been reported to reduce lung cancer risk (Godtfredsen, Prescott & Osler 2005) and the decline rate of FEV1 (Simmons et al. 2005), at least before the age of 55 years (Lange et al. 1989). Other results, by contrast, suggest that reduction improves respiratory symptoms, but has no effect on FEV1 decline (Bohadana et al. 2006, Pisinger & Godtfredsen 2007). A Finnish cohort study found that smokers with chronic bronchitis can improve their life expectancy by reducing their smoking (Pelkonen et al. 2006).

The extent to which light smoking affects the lungs is unclear. Cross-sectionally measured, light smoking has been reported to be associated with wheezing, shortness of breath, and cough, but not with chronic bronchitis (Pallasaho et al. 2002, Amigo et al. 2006). Some evidence has emerged that long-term passive smoking can cause chronic bronchitis (Radon et al. 2002, U.S. Department of Health and Human Services 2006, Mannino & Buist 2007), but whether light smoking can permanently reduce pulmonary function or cause COPD remains unknown**.**

Although light smokers are subjected to numerous health risks, studies have shown that they are rarely advised to quit smoking (Owen et al. 1995, Okuyemi et al. 2001). However, cessation interventions are important also for light smokers: after quitting, for example, smokers having consumed on average 7 CPD demonstrate similar relapse rates as heavy smokers (Choi et al. 2004). Studies on smoking cessation pharmacotherapies are mostly restricted to smokers consuming >10 CPD. Nicotine replacement therapy, bupropion, or varenicline are not recommended for light smokers, instead, behavioral therapies should be offered to them as well (Robinson, Schroeder & Moolchan 2006, Tønnesen et al. 2007, Cahill, Stead & Lancaster 2011).

2.3. Lung function

2.3.1. Measuring lung function

Spirometry is the most common and practical way to appropriately measure ventilatory function. It has been defined as “a physiological test that measures how an individual inhales or exhales volumes of air as a function of time” (Miller et al. 2005b). Subjects perform maximal and forced exhalations into a spirometer attached to a computer, which creates flow-volume and volume-time curves. Good cooperation and repeated measures are necessary to achieve reliable results (Miller et al. 2005b). If the testee suffers from, for example, chest pains or dementia, or has smoked, used alcohol, or bronchodilators prior to spirometry, the

results are easily suboptimal. The most important and informative values in spirometry are forced expiratory volume in one second (FEV1), slow vital capacity (VC) or forced vital capacity (FVC), and total lung capacity (TLC) (Viljanen 1982, Miller et al. 2005a).

In restrictive disorders, the ratio of FEV1/VC or FEV1/FVC is normal, while TLC is reduced (Pellegrino et al. 2005, Mannino et al. 2006). Instead, a reduced ratio of FEV1/VC or FEV1/FVC and a reduced FEV1 are characteristic of obstructive ventilatory disorders. To test the reversibility of airflow limitation, bronchodilators are administered to the testee, and lung function values are measured again after medication. Low values of FEV1/FVC and FEV1 even after inhalation of bronchodilators are typical of COPD, whereas in asthma the decrease is reversible (GOLD 2010).

Interpretation of spirometry measures is based on comparisons made between the testee and reference values derived from a healthy general population. Reference equations are calculated by taking into account subjects' sex, age, and height. Ideally, the reference values are derived from a population ethnically similar to the individual tested (Pellegrino et al. 2005). Reference equations vary according to race, and those used in the US and in Europe are different (Quanjer et al. 1993, Hankinson, Odencrantz & Fedan 1999). In Finland, use of national age- and height-adjusted reference values is recommended (Viljanen 1982, Sovijärvi et al. 2006).

2.3.2. Natural history of lung function

The natural history of lung function and the drastic effect of smoking on it were first explored in the 1970s in a sample of working men (Fletcher & Peto 1977). Studies with longer follow-up times and larger samples have increased the current knowledge of lung function decline and influencing factors (Xu et al. 1994, Mannino & Davis 2006, Kohansal et al. 2009).

FEV1 and FVC grow linearly until the age of 10-12 years, while during adolescence their increase is more complex (Wang et al. 1993). In males, lung function peaks around the age of 23 and in females this happens earlier and the plateau phase is longer (Kohansal et al. 2009). Men have greater absolute lung function measures than women, even when adjusted for height (Pellegrino et al. 2005). Many environmental factors during lung development, such as maternal smoking during pregnancy, passive and active smoking, asthma and airway hyperresponsiveness, and respiratory tract infections during childhood, may impair the achievement of maximal lung function (Bakke 2003) or result in a shortened plateau phase (Kerstjens et al. 1997). Interestingly, low socioeconomic status is associated with poor lung

function, even after adjustment for smoking, occupational risk factors, and race (Prescott, Lange & Vestbo 1999, Hegewald & Crapo 2007). An association between mental health problems and poor lung function has been observed in both adults (Goodwin et al. 2007) and children (Whitrow & Harding 2008); the underlying mechanism remains unclear.

After having reached maximum values, lung function begins to decrease. The decline rate of FEV1 varies between 20 and 40 ml/year (Kerstjens et al. 1997, Wise 2006, Kohansal et al. 2009), greater rates being observed in men (Kerstjens et al. 1997, Wise 2006). The above-mentioned environmental risk factors, particularly smoking, may speed up the age-related decline in lung function (Amara et al. 2001, Stern et al. 2007). Marked individual variation exists in the rate of lung function decline due to environmental exposure. Physically active individuals can maintain better lung function longer (Amara et al. 2001), whereas weight gain is associated with an enhanced decline in lung function (Chen, Horne & Dosman 1993, Wang et al. 1996, Thyagarajan et al. 2008). The effect of obesity is more pronounced in men than in women (Chen, Horne & Dosman 1993, Steele et al. 2009). Finally, lung function decline accelerates after the age of 65 years (Mannino & Davis 2006, Wise 2006). Even in healthy elderly individuals, multiple physiological modifications occur in the lungs with age, including loss of elastic recoil, decrease in oxygen diffusion capacity, and premature airway closure. These changes are similar to those observed in emphysema, and together with sarcopenia and anatomical changes of the chest wall, they result in smaller lung function values (Chan & Welsh 1998).

2.3.3. Lung function and smoking

Smoking is the most important factor enhancing the age-related decline in FEV1 (Kerstjens et al. 1997, Viegi et al. 2007). The risk of a significant obstruction increases with lifetime cumulative exposure to tobacco, independent of current smoking status (Burrows et al. 1977, Rennard & Vestbo 2006). Smoking cessation can normalize the accelerated decline in FEV1 (Anthonisen, Connett & Murray 2002, Bohadana et al. 2006, Kohansal et al. 2009), but smokers apparently should quit before middle age in order to attain this beneficial effect (Murray et al. 1998, Kohansal et al. 2009). Some studies report that reduction in daily cigarettes may slow down the decline in FEV1 (Lange et al. 1989, Simmons et al. 2005); others state that reduction has no effect on FEV1 (Bohadana et al. 2006, Pisinger & Godtfredsen 2007). Those who try to quit smoking, but relapse and become “intermittent

quitters", have slower loss of lung function than those who continue daily smoking (Anthonisen, Connett & Murray 2002).

Individual responses to tobacco smoke are different, and not all smokers eventually develop COPD. Multiple studies suggest that symptomatic smokers, i.e. those suffering from, for instance, chronic bronchitis, wheezing, or dyspnea, are more susceptible and have a steeper decline in FEV1 than asymptomatic smokers (Sherrill et al. 1991, Sherman et al. 1992, Tishler et al. 2002, de Marco et al. 2007, Kohansal et al. 2009). Women seem to be more susceptible to the effects of cigarette smoke and develop COPD more easily than men (Prescott et al. 1997, Langhammer et al. 2003, Foreman et al. 2011).

2.3.4. Heritability of lung function

Phenotypic differences between individuals are due to both environmental and genetic factors. Heritability is defined as the proportion of inter-individual variance explained by genetic factors. Because genetic and environmental effects vary across time and populations, heritability estimates are not constant. Genetic variance in a population can be divided into additive (A), dominant (D), and epistatic genetic effects. If the common effect of gene alleles at a locus is the sum of their individual effects, the effect is additive. Dominant effects are those deviating from purely additive effects, and epistatic effects refer to the interaction between alleles at different loci. Environmental factors can also modify genetic effects (GxE interaction). Heritabilities of different phenotypes can be estimated by comparing the similarities between monozygotic (MZ) and dizygotic (DZ) twins. The classic twin design is based on the assumption that MZ twins share all, while DZ twins share on average 50%, of their segregating genes (Evans, Gillespie & Martin 2002).

COPD sometimes clusters in families, and susceptibility to tobacco smoke differs between individuals (Sandford, Weir & Pare 1997, Wan & Silverman 2009). These observations have suggested that genetic effects control lung function. To estimate its heritability, twin and family studies have analyzed spirometry values. Lung function has been shown to be influenced by both genetic and environmental factors; however, the reported effects of aging, sex, and smoking on heritability are controversial. Multivariate-adjusted heritability estimates are 10-67% for FEV1, 40-59% for FVC, and 45% for the FEV1/FVC ratio (McClearn et al. 1994, Wilk et al. 2000, Palmer et al. 2001, Hallberg et al. 2010).

In the 1980s, some twin and family studies found that no significant lung function heritability exists after adjustment for body habitus or height (Hubert et al. 1982, Lebowitz,

Knudson & Burrows 1984, Ghio et al. 1989), while others reported high heritability estimates (Lewitter et al. 1984, Redline et al. 1987). Results obtained in segregation analyses of lung function were also controversial; both polygenic genetic factors and environmental factors (Chen et al. 1996, Givelber et al. 1998) as well as a major gene effect (Wilk et al. 2000) have been suggested to control FEV1. Studies using quantitative genetic modeling have consistently reported that the observed variance in spirometry values can be decomposed into additive genetic effects and environmental effects (Palmer et al. 2001, Zhai et al. 2007, Hallberg et al. 2010). Aging and smoking, however, influence the heritability estimates of lung function, and taking into account the effect of smoking has either increased or decreased heritability estimates (Coultas et al. 1991, McClearn et al. 1994, Zhai et al. 2007). Results on sex differences in lung function are also controversial (McClearn et al. 1994, Hallberg et al. 2010).

Limitations of lung function heritability studies include cross-sectional study design and inclusion of both smokers and nonsmokers, which makes it difficult to control for the smoking-gene interaction. Lung function is likely to be influenced by a substantial GxE interaction (Silverman et al. 1998, Wilk et al. 2000, Zhai et al. 2007), and smoking has been shown to modify the expression of multiple genes (Zeller et al. 2010). Further, studies have assessed pulmonary function at only one time-point, although heritability estimates may change over time as a result of aging and changing environmental exposures. Repeated spirometry measurements have been used only in one study, which estimated the heritability of the lung function decline rate (Gottlieb et al. 2001).

Candidate gene studies, performed on both subjects with respiratory diseases and general populations, have identified over 100 genes suggested to control pulmonary function. The reported associations have not, however, been consistent across different studies. A recent meta-analysis re-analyzed the reported candidate single-nucleotide polymorphisms (SNPs) of lung function in a large population sample. All previously reported associations were found to be nonsignificant, and the strongest relations with FEV1 were in the phosphodiesterase 4D (PDE4D) gene and the SERPINA1 gene encoding the alpha-1 antitrypsin protein (AAT) (Obeidat et al. 2011). In the lungs, PDE4 regulates smooth muscle contractility, and its expression is associated with chronic inflammation (Soto & Hanania 2005). A mutation in SERPINA1 leads to AAT deficiency, which has been known for decades to be the most important genetic disorder predisposing to COPD (Wan & Silverman 2009).

As shown by heritability studies, pulmonary function is likely to result from an interaction between multiple genes and the environment. In recent years, GWAs have become a popular means of examining the genetic background of complex diseases. By investigating the genomic variants of hundreds of thousands of subjects, GWAs have identified multiple SNPs associated with FEV1 and the FEV1/FVC ratio (Wilk et al. 2009, Hancock et al. 2010, Repapi et al. 2010). Some of these associations have been consistently replicated, while others only in certain populations. The identified SNPs have been located in different chromosomes, interestingly one also in chromosome 15, near the nAChR gene (Hancock et al. 2010). However, these variants have small effects and explain <1% of the variation in lung function. Thus, the genetic pathways influencing lung function mostly remain unidentified.

2.4. Chronic bronchitis

2.4.1. Definition and history

Chronic bronchitis is defined as chronic cough and mucus production from the airways for at least three months per year for at least two successive years. Inflammation of the mucosal surface, mucous gland hyperplasia, and changes in the cell proportions of the airways are characteristic pathological changes in chronic bronchitis (Barnes et al. 2002). The diagnosis is clinical and is based on the presence of symptoms, which are often screened with standardized questionnaires, such as the British Medical Research Council (MRC) series of questions, already published in 1960 (British Medical Research Council 1960). Chronic bronchitis is a reversible state (Willemse et al. 2004), but it can also affect patients with COPD and permanent airflow limitation.

Chronic bronchitis was first defined in the CIBA Guest Symposium in 1959, where the term ‘chronic’ was specified as “occurring on most days for at least three months of the year for at least two consecutive years” (Fletcher et al. 1959). The common term COPD was introduced in 1965 (Petty 2006); however, ‘chronic bronchitis’ and ‘emphysema’ were commonly used as diagnoses until the 1970s. Since then, use of the term ‘COPD’ started to increase and it largely replaced those terms (GOLD 2010). Today, chronic bronchitis refers to long-standing symptoms, and emphysema is a pathological term referring to alveoli destruction (GOLD 2010). In Finland, 'chronic bronchitis' and 'emphysema' were occasionally used as diagnoses for COPD still in the 1980-1990s.

2.4.2. *Smoking and other risk factors*

Smoking is the most important risk factor for chronic bronchitis (Sethi & Rochester 2000), and the disease has been estimated to affect as many as 40% of smokers (Pelkonen et al. 2006). Although the prevalence of chronic bronchitis increases with age and pack-years of smoking, the disease also affects young adults, even those under 20 years of age (Cerveri et al. 2001, de Marco et al. 2007, Hamari et al. 2010). However, many smokers become accustomed to their respiratory symptoms and consider themselves healthy (Toljamo et al. 2010). Recovery from chronic bronchitis may be achieved after smoking cessation (Brown et al. 1991, Willemse et al. 2004) but smoking reduction does not appear to have a similar beneficial effect (Simmons et al. 2005, Bohadana et al. 2006). No evidence exists whether light smoking can cause chronic bronchitis. A Finnish study found the risk of chronic bronchitis to be increased only among smokers consuming >5 CPD (Pallasaho et al. 1999). However, other studies have reported that even passive and occasional smoking can cause chronic bronchitis (Radon et al. 2002, Mannino & Buist 2007, Yin et al. 2007, Hamari et al. 2010).

In addition to smoking, other known risk factors for chronic bronchitis are asthma, atopy (Terho, Koskenvuo & Kaprio 1995), history of respiratory tract infections (Lange et al. 2003), farming, industrial work (Laasonen & Uitti 2001, Eduard, Pearce & Douwes 2009), outdoor work (Kotaniemi et al. 2003), exposure to biomass fuels, and occupational exposure to gases, dusts, or fumes (Lange et al. 2003, Eduard, Pearce & Douwes 2009, Salvi & Barnes 2009). Physical activity slows down age-related pulmonary function decline regardless of smoking status (Pelkonen et al. 2003, Garcia-Aymerich et al. 2007), and, to some extent, seems to protect from chronic bronchitis (Kujala et al. 1996). Genetic factors independent of smoking play a role in the susceptibility of developing chronic bronchitis (Svartengren et al. 2009).

2.4.3. *Epidemiology of chronic bronchitis*

The European Community Respiratory Health Survey (ECRHS) has estimated the prevalence of different respiratory diseases in young adults of 35 centers from 16 countries. Among subjects aged 20-44 years, the median prevalence of chronic bronchitis was 2.6%, with wide variations across countries. Men were affected significantly more often than women, and 73% of those affected were current smokers (Cerveri et al. 2001). Similar questionnaires were sent

to subjects aged 45-69 years in Australia, where chronic bronchitis was reported by 12% (Abramson et al. 2002). A French population survey, where the mean age of participants was 51.1 years, reported a chronic bronchitis prevalence of 4.1% (Huchon et al. 2002). The Copenhagen City Heart Study among men and women aged over 65 found chronic bronchitis prevalences of 19% and 13%, respectively (Lange et al. 2003). The World Health Organization (WHO) estimated the prevalence of chronic bronchitis to vary between 7% and 17% across countries (Global Alliance against Chronic Respiratory Diseases 2007). These results reflect the geographic variation in chronic bronchitis as well as its increasing prevalence with age. Higher disease rates among men than women are mainly due to higher smoking prevalence among men (Cerveri et al. 2001); however, the sex-related differences in the prevalence of both chronic bronchitis and smoking are diminishing (Helakorpi, Prättälä & Uurtela 2008, Chilcoat 2009).

The prevalence of chronic bronchitis, similarly to that of smoking, has decreased considerably since the 1960s in Finland (Huhti 1965, Terho et al. 1987, Reijula et al. 1990, von Hertzen et al. 2000). In the 1990s, chronic bronchitis was estimated to affect 400 000 subjects in Finland, equivalent to 10% of the adult population, with substantial regional and sex-related differences (Laitinen & Koskela 1999). The FinEsS study assessing prevalence rates and risk factors of airway disorders in Finland, Estonia, and Sweden, recently found the self-reported prevalence of chronic productive cough to be 12% in Helsinki and 11% in Lapland among 12 695 subjects aged 20-64 years. However, the prevalence of physician-diagnosed chronic bronchitis was only 3.4% in Helsinki and 2.9% in Lapland (Kotaniemi et al. 2002). The prevalence of self-reported chronic bronchitis was significantly higher in northern Finland than in northern Sweden (7%), whereas the prevalence of physician-diagnosed chronic bronchitis was higher in northern Sweden (Lindström et al. 2001). These numbers are in line with the view that chronic bronchitis is often underdiagnosed.

A 40-year cohort study in a Finnish rural population estimated the cumulative incidence of chronic bronchitis to be 42% in continuous smokers, 26% in former smokers, and 22% in never smokers. The symptoms were usually persistent in smokers, whereas temporary in others (Pelkonen et al. 2006). No other long-term studies exist on the incidence of chronic bronchitis, and the above-mentioned estimates were derived from a population where most of the subjects were farmers.

2.4.4. *Effect on pulmonary function*

Smokers with chronic bronchitis have an excess risk for COPD relative to asymptomatic smokers (Lange et al. 2003, de Marco et al. 2007, Guerra et al. 2009). However, even without the development of COPD, chronic bronchitis can affect pulmonary function. Chronic productive cough increases the risk of asthma (Troisi et al. 1995, Huovinen et al. 1999) and shortness of breath in exercise (Kotaniemi et al. 2003). Chronic bronchitis has also been shown to be associated with decreased FEV1 values (Vestbo, Prescott & Lange 1996, Pelkonen et al. 2006, Pelkonen 2008, Eduard, Pearce & Douwes 2009) and an increased prevalence of obstructive sleep apnea (Larsson et al. 2001). The impairment of pulmonary function is, however, reversible and can be slowed down by smoking cessation (Willemse et al. 2004).

2.4.5. *Other health effects*

Chronic bronchitis is associated with poor quality of life (Doll et al. 2002), depression, and anxiety (Wagena et al. 2005). COPD patients with chronic bronchitis report more wheezing, nasal and ocular symptoms, and exacerbations than those without chronic bronchitis but with similar lung function (Kim et al. 2011). Chronic bronchitis increases the risk of coronary heart disease, and this association remains significant after the effect of smoking has been taken into account (Jousilahti et al. 1996). Chronic bronchitis is related to increased all-cause mortality, even in nonsmokers, in subjects aged <50 years, and in those without the presence of airflow obstruction (Lange et al. 2003, Pelkonen et al. 2006, Guerra et al. 2009). COPD patients who suffer from chronic bronchitis symptoms have a higher mortality risk than asymptomatic patients (Ekberg-Aronsson et al. 2005).

2.5. *Chronic obstructive pulmonary disease*

2.5.1. *Definitions*

According to the Global Initiative for Obstructive Lung Disease (GOLD) guidelines and the common guidelines of American Thoracic Society (ATS) and European Respiratory Society (ERS), COPD is a “preventable and treatable disease with some extrapulmonary effects that may contribute to the severity in individual patients. Its pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lungs to noxious

particles or gases" (Celli, MacNee & ATS/ERS Task Force 2004, GOLD 2010). The clinical picture of the disease depends on whether the inflammation site is mainly in the small airways, leading to obstructive bronchiolitis, or in lung parenchyma, leading to emphysema. The airflow limitation is caused by a mixture of these pathological changes, and sometimes asthma or chronic bronchitis worsens the obstruction (Mannino & Buist 2007).

The common guidelines of GOLD, ATS, and ERS define COPD as post-bronchodilator FEV1/FVC ratio < 0.70 in spirometry. The stage of the disease is determined by the value of FEV1, which is ≥80% of predicted in mild (stage I) COPD, ≥50% but <80% of predicted in moderate (stage II) COPD, and ≥30% but <50% of predicted in severe (stage III) COPD. In very severe (stage IV) COPD, the patient has an FEV1 <30% of predicted, or <50% plus chronic respiratory failure (GOLD 2010). Even though these criteria are widely used, they have been criticized because they seem to result in overdiagnosis in the elderly and underdiagnosis in young subjects (Hardie et al. 2002, Medbo & Melbye 2007). The British Thoracic Society (BTS) uses a criterion of FEV1<80% of predicted in addition to FEV1/FVC<0.7 to define COPD. Use of an additional criterion, FEV1<lower limit of normal (LLN) or FEV1<80% of expected, is increasingly recommended because it reduces age-related biases and the number of false-positive diagnoses (Swanney et al. 2008, Vollmer et al. 2009). The LLN cutoff is defined as being below the lower fifth percentile of a large healthy reference group. Calculating the LLN values, which take into account the subject's age and height, thus requires availability of reliable reference equations. Using the LLN for FEV1/FVC instead of the fixed ratio<0.7 is also an acceptable way to avoid false-positive cases (Swanney et al. 2008).

Although reduced lung function is a common feature among COPD patients, several different phenotypes of the disease exist. The extent of airway inflammation and emphysema has long been known to vary among patients. However, the frequency of exacerbations, the severity of gas exchange impairment, and airway hyperresponsiveness, for example, also contribute to different COPD phenotypes. The expression of disease components among patients can be used to classify them into subgroups, or phenotypes, who respond to therapy in different ways. Researchers aim at identifying phenotypes that can be offered specific therapies (Han et al. 2010).

2.5.2. *Risk factors*

Many environmental factors causing chronic bronchitis can eventually also result in COPD. The single most important cause of COPD is tobacco smoking, which accounts for about 75% of COPD-related mortality in Western countries. In countries with lower income, 40% of COPD mortality has been estimated to be caused by tobacco, exposure to biomass fuels being the major risk factor (Mannino & Buist 2007, Salvi & Barnes 2009). Airway hyperresponsiveness, childhood respiratory infections, and a family history of asthma are important risk factors of COPD, particularly among subjects aged under 45 years (de Marco et al. 2011). Occupational exposure to dusts and fumes, air pollution, and passive smoking may also contribute to the development of COPD (Mannino & Buist 2007). Occupational exposure has been estimated to account for 15% of the burden of COPD with, however, large regional variance (Balmes et al. 2003, Salvi & Barnes 2009). In Finland, the attributable fraction of occupational mortality of all COPD deaths was 12% when exposure to dust and welding fumes at work was examined (Nurminen & Karjalainen 2001). Exposure to dusts is common in, for example, farmers, and their mortality due to respiratory diseases has been 40-50% higher than the average in Finland (Notkola, Husman & Laukkanen 1987). Physical activity in COPD patients can reduce mortality, but has no influence on disease progression (Chavannes et al. 2002, Garcia-Aymerich et al. 2007). Women seem to be more susceptible than men to the effects of smoking, resulting in more severe symptoms of COPD (Langhammer et al. 2003, Sorheim et al. 2010). Low educational and socioeconomic status are independent risk factors for COPD (Prescott, Lange & Vestbo 1999, Kanervisto et al. 2011).

2.5.3. *Epidemiology of COPD*

Because COPD is rarely diagnosed in its early phase, national registries generally underestimate its prevalence. The WHO estimates that COPD affects 210 million people worldwide (Global Alliance against Chronic Respiratory Diseases 2007). Population studies using spirometry have estimated COPD prevalence to be 5-15% in Europe and in the US (Mannino et al. 2000, Pena et al. 2000, Lundbäck et al. 2003). A large study among various geographic sites reported the prevalence of GOLD stage II or higher COPD to be 10.1% among subjects aged ≥40 years (Buist et al. 2007), whereas the prevalence was 8.3% when using FEV1/FVC<LLN as the disease definition (Vollmer et al. 2009). In all studies, the

prevalence rates have been highest among elderly smokers, and the majority of disease cases have had no previous diagnosis of COPD. Reasons for the underdiagnosis of COPD include patients' adjustment to current symptoms as well as the lack of symptoms in the early disease phase. Moreover, an estimated 10% of COPD patients actually do not fulfill the spirometry criteria of COPD. This can occur if both FEV1 and FVC are reduced, and their ratio is normal (Wan et al. 2011).

Few studies have estimated the incidence of COPD diagnosed with spirometry. Among elderly subjects with respiratory symptoms, the ten-year cumulative incidence of COPD was reported to be 13.5% (Lindberg et al. 2005). Another study found the nine-year cumulative incidence of COPD to be 6.1% among adults aged 18-74 years, and the risk was 10-fold greater among those aged over 45 years than in those younger than 45 years (Johannessen et al. 2005). Young age does not, however, protect against the disease; among subjects aged 20-44 years and with a normal lung function, a large international cohort found the ten-year cumulative incidence of COPD to be 2.8%. In that study, the cumulative incidence was 4.6% among those aged 40-44 years (de Marco et al. 2007). In Finland, the ten-year cumulative incidence of COPD, defined as FEV1<60% of predicted, was 2.5% in a rural population of subjects aged 40-64 years (Huhti & Ikkala 1980). A 40-year follow-up study reported the cumulative incidence of COPD to be 32% among continuous smokers, 14% in former smokers, and 12% in never smokers (Pelkonen et al. 2006).

In the 1990s, 200 000 Finns (5% of the population) were estimated to suffer from symptomatic COPD, whereas the number of asymptomatic patients was unknown (Laitinen & Koskela 1999). One study using spirometry reported prevalence rates of 12.5% and 3.0% for elderly males and females, respectively (Isoaho et al. 1994). Recently, the FinEsS study estimated that the prevalence of COPD according to GOLD criteria was 9.4% in northern Finland (15.6% in men and 3.7% in women). By applying the BTS criteria, the prevalence decreased to 5.4%. (Kotaniemi, Sovijärvi & Lundbäck 2005). Of the affected subjects, less than 25% had been diagnosed previously, and only one-third reported chronic productive cough. Another recent Finnish study with a larger number of participants reported the prevalence of COPD, defined as FEV1/FVC<LLN, to be 4.3% and 3.1% among men and women, respectively, and the prevalence had not changed between 1980 and 2000 (Vasankari et al. 2010). Since the 1990s, hospitalizations due to COPD have decreased, while COPD-related mortality has remained unchanged (Kinnula et al. 2011).

Worldwide, COPD prevalence and related mortality are increasing rapidly, mainly due to increased smoking and use of biomass fuels in low- and middle-income countries. Both of these risk factors particularly affect women; today, women suffer from COPD almost as often as men (Global Alliance against Chronic Respiratory Diseases 2007, Salvi & Barnes 2009). In 2004, COPD was the fourth leading cause of death worldwide, and the WHO predicts that it will become the third leading cause of mortality by 2030 (World Health Organization 2004). In high-income countries, COPD is the major chronic disease for which mortality is increasing. In the US, for instance, death rates from COPD have doubled between 1970 and 2002 (Global Alliance against Chronic Respiratory Diseases 2007).

2.5.4. Association between COPD and smoking patterns

Previously, COPD was estimated to develop in 20-25% of smokers (Fletcher & Peto 1977, Mannino 2003). Due to underdiagnosis of COPD, these estimates are, however, too small (Rennard & Vestbo 2006, Mannino & Buist 2007). Recent studies have shown that the actual proportion of affected smokers is greater (Lundbäck et al. 2003, Lokke et al. 2006, Pelkonen et al. 2006).

Regarding pulmonary function, no safe level of smoking has been suggested to exist. Although significant and long-standing exposure to environmental tobacco smoke probably accounts for some of the COPD cases among never smokers (Yin et al. 2007), it remains unclear whether moderate levels of passive smoking can result in permanent airflow obstruction (Coultas 1998, U.S. Department of Health and Human Services 2006). Neither is there evidence that regular light smoking causes COPD. One study reported that cross-sectionally measured light smoking among subjects aged over 45 years was associated with COPD by using the GOLD criteria; however, no association was found when FEV1<80% was considered as an additional criterion (Lundbäck et al. 2003). Reducing heavy smoking substantially may reduce mortality as well as slow down the decline of FEV1 in chronic bronchitis patients (Lange et al. 1989, Simmons et al. 2005, Pelkonen et al. 2006). Nevertheless, hardly any studies exist on the effect of smoking reduction on COPD development. Reducing heavy smoking by 50% does not change the risk for hospital admissions due to COPD (Godtfredsen et al. 2002). Early quitting, by contrast, may protect from COPD development (Lokke et al. 2006).

2.5.5. *Health effects*

COPD patients often suffer from comorbidities, which are partly caused by aging and smoking and partly by the extrapulmonary effects of COPD itself (Agusti 2005). COPD patients are at increased risk for cardiovascular diseases, osteoporosis, diabetes, respiratory infections, and lung cancer (Young et al. 2009, GOLD 2010). Weight loss, nutritional abnormalities, and muscle waste are common extrapulmonary effects of COPD (Schols & Wouters 2000), causing disability and poor exercise performance. These effects have been suggested to be mediated by tissue hypoxia, oxidative stress, increased metabolic rate, and systemic inflammation (Agusti 2005). Increased serum C-reactive protein (CRP) levels and microalbuminuria have been detected in COPD patients (Han 2010). Psychiatric problems, such as depression (Hanania et al. 2011), sleep disorders, and anxiety (Eisner et al. 2010, GOLD 2010) are also associated with COPD.

In addition to comorbidities, exacerbations are another feature characteristic of COPD. Exacerbation is an acute worsening of the patient's condition, usually characterized by increased respiratory and systemic symptoms and an increased need for medication (Rodriguez-Roisin 2000). Frequent and severe exacerbations are associated with poorer survival (Soler-Cataluna et al. 2005). Other predictors of mortality in COPD patients include dyspnea, exercise performance, and body mass index (BMI), whereas the degree of obstruction is only weakly associated with mortality risk (Celli 2010).

2.5.6. *Treatment of COPD*

Because the only way to slow down the progression of COPD in its early phase is to avoid exposure to risk factors, smoking cessation is the primary intervention in COPD. Among patients with chronic respiratory failure, long-term oxygen therapy increases survival. Use of noninvasive ventilation (NIV), positive or negative pressure to support breathing, is recommended in acute excerbations among patients with moderate or severe COPD. Use of NIV reduces dyspnea, length of hospitalizations, readmissions, and mortality (Celli, MacNee & ATS/ERS Task Force 2004, GOLD 2010). Medications may relieve symptoms, increase exercise capacity, and reduce the number of exacerbations, but cannot normalize the enhanced decline in lung function. Pharmacological treatment of COPD consists of inhaled anticholinergics and beta agonists, theophylline, and corticosteroids (Celli, MacNee & ATS/ERS Task Force 2004).

Narrowing of the airways, caused by smooth muscle hyperplasia and an increase in connective tissue, is the main reason for airflow limitation in COPD. Loss of elastic recoil and alveoli destruction also contribute to the obstruction (Barnes et al. 2002). In COPD, the neuronal parasympathetic activity in the airways is pathologically enhanced, resulting in an excess release of acetylcholine. This contracts smooth muscle cells and increases mucus production and airway hyperresponsiveness (Belmonte 2005). Enhanced parasympathetic activity is the main reversible component of bronchoconstriction in COPD and has been treated with anticholinergic compounds for decades. Anticholinergics have few adverse effects and are well tolerated (Gross 2006). In Finland, there are currently two anticholinergics in use: short-acting ipratropium bromide and long-acting tiotropium bromide. Use of ipratropium peaked in the 1990s, but has now been largely replaced by the more effective tiotropium, which came onto the market in 2002. Patients with severe COPD have been eligible for a special reimbursement for tiotropium purchases since 2004. Oxitropium was withdrawn from the market in 2002 (*Finnish Statistics on Medicines,* 1995-2008).

The sympathetic nervous system controls airway smooth muscle tonus through $beta_2$ receptors. $Beta_2$ agonists stimulate these receptors as well as decrease parasympathetic activity, resulting in smooth muscle relaxation (Tashkin & Fabbri 2010). Short-acting $beta_2$ agonists are used both alone and in combination with ipratropium to relieve acute symptoms. Long-acting $beta_2$ agonists are used to treat stable COPD, however, they are not as effective as tiotropium (Rodrigo, Nannini & Rodriguez-Roisin 2008). $Beta_2$ agonists' usual adverse effects include palpitations and tremor (Tashkin & Fabbri 2010).

International guidelines recommend using short-acting bronchodilators when needed for mild COPD, and adding a long-acting bronchodilator, either tiotropium or a $beta_2$ agonist, to treatment when the disease progresses to stage II. Inhaled corticosteroids are recommended for those with frequent exacerbations (GOLD 2010). Finnish Current Care Guidelines for COPD treatment are similar, except that they recommend tiotropium over long-acting $beta_2$ agonists for COPD stage II-IV (Kinnula et al. 2009). A large randomized control study recently found that tiotropium is more effective than long-acting $beta_2$ agonist in preventing exacerbations (Vogelmeier et al. 2011).

Roflumilast is a new oral medication that is particularly effective in COPD patients suffering from chronic bronchitis (Rennard et al. 2011). It is used together with bronchodilators. In Finland, COPD patients with chronic bronchitis, frequent exacerbations,

and FEV1<50% have been eligible for a basic reimbursement for roflumilast purchases since 2011.

2.5.7. The Finnish medication reimbursement system

Finland's Social Insurance Institution (SII) contributes to patients' medication purchases from a pharmacy. A basic reimbursement, covering 42% of medication price, is granted for all prescribed purchases. A special reimbursement to cover 72-100% of medication expenses can be allocated if the patient has been diagnosed by a specialist, and the disease fulfills certain severity criteria. In addition to the amount remaining, the patient pays a fixed deductible at the pharmacy. It is most economical to buy the maximum amount of the reimbursed drug, a three-month requirement, at one time because only one fixed deductible is paid irrespective of the number of medication refills (Martikainen & Rajaniemi 2002). The next purchase can be made when the previous amount has been almost depleted (The Social Insurance Institution of Finland 2010). Special reimbursement eligibilities have been allocated for asthma since 1973. In 1985, also other chronic obstructive lung diseases were included, with medications for severe COPD reimbursed. The percentage covered was 90% from 1985 to 1992, 80% during 1993, 75% from 1994 to 2006, and 72% from 2007 onwards (Valtioneuvoston asetukset vaikeiksi ja pitkäaikaisiksi arvioitavista sairauksista, joiden lääkehoidon kustannuksista sairasvakuutuslain perusteella korvataan 72 tai 100 prosenttia).

Until 2007, a special reimbursement for COPD medications was allocated if the patient had an FEV1<40% of predicted, or arterial pCO_2 constantly >6.5kPa (stage III-IV COPD). Since then, FEV1<50% was considered severe enough if the patient suffered from exacerbations during bronchodilator treatment. In the 2011 updated criteria, arterial pCO_2 is no longer included. (Finland's Social Insurance Institution 2010). The SII tracks reimbursement eligibilities and medications for asthma, COPD, and other obstructive lung diseases together (Laitinen & Koskela 1999), and has collected exact diagnoses only since 2000. Before that, diagnoses were registered occasionally, and COPD was often diagnosed as 'emphysema' or 'chronic bronchitis' (Kotaniemi 2006, Laitinen & Koskela 1999).

2.5.8. Genetics of COPD

AAT deficiency, identified in 1963, is the most important known genetic risk factor for COPD (Wan & Silverman 2009). It is caused by inheritance of two protease inhibitor deficiency alleles from the AAT gene locus on chromosome 14. The prevalence of AAT

deficiency is highest in Europe and North America, 1/2000-7000, accounting for 1-2% of COPD cases. Smokers with AAT deficiency usually develop severe COPD as young adults, whereas nonsmoker carriers may be asymptomatic (Barker et al. 1997, Wan & Silverman 2009). Variants of the AAT encoding gene may explain why some subjects develop COPD more easily, although they are not deficiency allele carriers (Obeidat et al. 2011). Another common autosomal recessive disorder possibly associated with COPD is cystic fibrosis, the result of a mutation in the cystic fibrosis transmembrane conductance regulator gene. The disease causes bronchiectasis and chronic bacterial lung infections, which may predispose to COPD (Sandford, Weir & Pare 1997). In most subjects, however, the clinical picture of COPD is likely to arise from an interaction between multiple genes and environmental factors.

Multiple candidate gene studies have found polymorphisms associated with COPD. Studies in large population samples, for instance, have demonstrated that matrix metalloproteinase gene (MMP12) (Hunninghake et al. 2009, Haq et al. 2011) and SERPINE2 gene encoding thrombin, urokinase, and plasmin inhibitors (Zhu et al. 2007) are associated with COPD. MMP12 is produced in the lungs by macrophages. Its amount is known to increase due to smoking, whereas the role of the SERPINE2 gene in the lungs is unclear. However, the results of candidate gene studies are inconsistent, and meta-analyses have also produced discrepant findings (Hersh et al. 2005, Smolonska et al. 2009, Castaldi et al. 2010). SERPINA1 gene, encoding alpha-1-antitrypsin, remains the only known gene with a consistent and reliable association with COPD (Obeidat et al. 2011).

GWAs have identified multiple genomic variants associated with COPD. Many such variants are associated also with lung function, e.g. those near the hedgehog interacting protein (HHIP) region (Pillai et al. 2009, Cho et al. 2010, Hancock et al. 2010, Repapi et al. 2010). By testing the multiple SNPs previously identified as associating with FEV1 and FEV1/FVC (Wilk et al. 2009, Hancock et al. 2010, Repapi et al. 2010), a recent meta-analysis found that three loci in chromosomes 4, 5, and 6 were associated also with COPD susceptibility (Castaldi et al. 2011). Variants in the nAChR gene on chromosome 15, in turn, are associated with COPD, lung cancer, and emphysema (Pillai et al. 2009, Lambrechts et al. 2010) as well as with nicotine dependence and smoking behavior (Thorgeirsson et al. 2010). A cohort study found that after adjustment for smoking, the nAChR polymorphisms independently increased the risk of all these tobacco-related diseases (Kaur-Knudsen et al. 2011). One possible explanation for this is that the receptor gene variants increase the

cholinergic activity in the lungs, resulting in excess mucus production, smooth muscle contraction, and perhaps also inflammatory cell recruitment (Wessler, Kirkpatrick & Racke 1998).

COPD prevalence is increased in subjects with lung cancer, regardless of smoking status and history of pack-years (Young et al. 2009). The inflammatory and immune-modulating pathways seem to be similar in these diseases (Young & Hopkins 2011). A variant in chromosome 5, previously reported to be associated with lung cancer, was recently found to also be associated with airflow limitation and emphysema independent of smoking (Wauters et al. 2011), supporting the view that these pulmonary diseases share some common genetic background.

The newly identified susceptibility loci explain only a small proportion of the genetics of COPD. Individual susceptibility for the disease cannot be tested, and smoking remains the most important preventable risk factor for COPD. However, new discoveries may provide novel insights into disease pathophysiology. It is possible that 'susceptible smokers' are the ones who carry these risk alleles in their genome.

2.6. Summary

Although the associations of moderate and heavy smoking with chronic bronchitis and COPD are well established, studies on the pulmonary effects of reduced smoking, light smoking, and other low-rate smoking patterns are few. Light and occasional smoking are increasing in many countries, however, the phenomenon of light smoking has not been investigated earlier in a Finnish population. Finnish health surveys have shown that smokers consume on average less cigarettes daily than earlier. Also the proportion of former smokers is increasing. When examining the health effects of different low-rate smoking patterns, a longitudinal study design is an advantage, allowing observation of changes in smoking patterns and subsequent disease development rates.

COPD is defined as a decreased lung function, with lung function also being an important independent marker of increased morbidity and mortality. Lung function declines physiologically throughout adulthood, and COPD incidence accumulates in older age. In addition to environmental factors that predispose to COPD, multiple other determinants affect lung function. Recent research has identified genetic variants associated with both lung function and COPD. However, the proportions of genetic and environmental factors affecting lung function vary across time and populations. The heritability of lung function has not been

evaluated earlier in a Finnish population, nor has it been assessed longitudinally or by excluding the effect of smoking.

The trend to reduced smoking patterns potentially has a high public health impact. Because COPD morbidity is increasing globally, and the pulmonary effects of such smoking patterns are partly unknown, research on this subject at a population level is warranted. Further, as COPD manifests as decreased lung function, understanding the genetic and environmental determinants of lung function helps to target interventions to prevent COPD. Moreover, recognizing factors that support maintenance of good lung function might also help to prevent excess morbidity and mortality.

3. Aims of the study

The aim of this thesis was to investigate longitudinal smoking patterns, especially light smoking and various changing smoking patterns, and their associations with pulmonary diseases. In addition, this study aimed to assess genetic and environmental factors independent of smoking that influence lung function. The main aim was to demonstrate that regarding pulmonary morbidity no safe level of smoking exists. Specific aims of this thesis were as follows:

1. To investigate the prevalence and consistency of different smoking patterns in a Finnish population by focusing on the subgroup of daily light smokers, and to evaluate the characteristics of Finnish light smokers (Study I).

2. To explore the heritability of lung function with repeated spirometry measurements by excluding the effect of smoking (Study III).

3. To estimate the incidence and risk of chronic bronchitis according to various smoking patterns by taking into account changes in smoking behaviors over a 6-year period (Study II).

4. To estimate the incidence and risk of COPD over a 27-year follow-up according to changes in smoking patterns over a 6-year period (Study IV).

4. Methods

4.1. Participants

4.1.1. The Older Finnish Twin Cohort

The Older Finnish Twin Cohort was established in 1974 to examine the genetic, environmental, and psychosocial determinants of health behaviors and chronic diseases. The cohort was compiled from the Central Population Registry by identifying as twin candidates sets of persons born on the same day, with the same surname at birth, of the same sex, and born in the same local municipality. The cohort included virtually all same-sex twin pairs born in Finland before 1958 with both members still alive in 1967 (Kaprio & Koskenvuo 2002). Questionnaires were distributed in 1975, 1981, and 1990, with response rates of 89%, 84%, and 77%, respectively. The 1981 questionnaire was delivered to all twins still alive in the cohort, whereas in 1990 the questionnaire was sent only to pairs born in 1930-1957, with both co-twins resident in Finland in 1987, if they had replied to at least one of the previous surveys. Data on subjects who participated in both the 1975 and 1981 surveys (n=21 609) were used in Studies I, II, and IV. Of these subjects, 11 015 participated again in 1990, and also data from this measurement point were used in Studies I and II.

4.1.2. The FITSA substudy

The Finnish Twin Study on Aging (FITSA) is a study of genetic and environmental effects on the disablement process in older female twins. The participants were recruited from the older Finnish Twin Cohort on the basis of age, sex, and zygosity (Tiainen et al. 2004). Invitations were sent in the year 2000 to 414 female twin pairs (178 MZ pairs, 212 DZ pairs, and 24 pairs of unknown zygosity, XZ) born in 1924-1937. Inclusion criteria were the willingness of both individuals in the pair to participate, ability to walk 2 km, and ability to travel independently to the research center. Eventually, 217 twin pairs (98 MZ, 106 DZ, and 13 XZ pairs) participated in the laboratory examinations and filled in questionnaires. At the follow-up in 2003, all baseline participants were invited to participate, and individual twins could attend even if their co-twins refused. In 2003, 419 individuals completed questionnaires and 313 participated in the laboratory measurements. Among never-smoking participants (n=374), those who attended spirometry at least at baseline (n=339) composed the sample of Study III. The attendance rates of never smokers in the FITSA study are described in Figure 1.

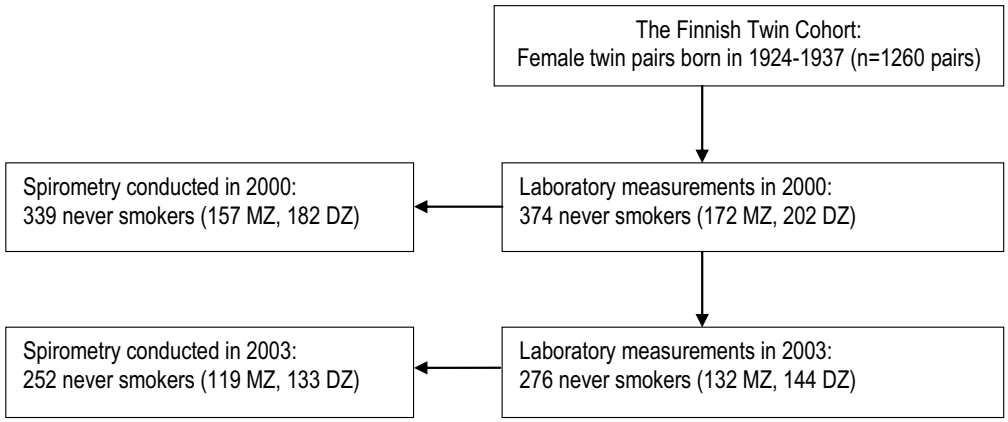

Figure 1. Participation rates of never smokers in the FITSA study.

4.2. Measurements

4.2.1. Smoking patterns

4.2.1.1. Twin cohort questionnaires

In the 1975 and 1981 questionnaires, smoking status was defined with a series of questions. Figure 2 presents the classification of smoking status and the exact questions asked. Never smokers were first separated from ever smokers and then occasional and former smokers were identified. Those who were classified as current smokers responded also to the question defining average daily cigarette consumption: ‘‘How many cigarettes do you smoke daily on average?’’ Former smokers were asked how old they were when they quit smoking and how many cigarettes they used to smoke daily. The amount categories were as follows: <5, 5–9, 10–14, 15–19, 20–24, 25–39, and >40. In this study, 1975 and 1981 current smokers were further collapsed into three groups: daily light (<5 CPD), daily moderate (5-19 CPD), and daily heavy (≥20 CPD) smokers. The term ‘low-rate smoking patterns’ includes in this thesis both light and occasional smoking.

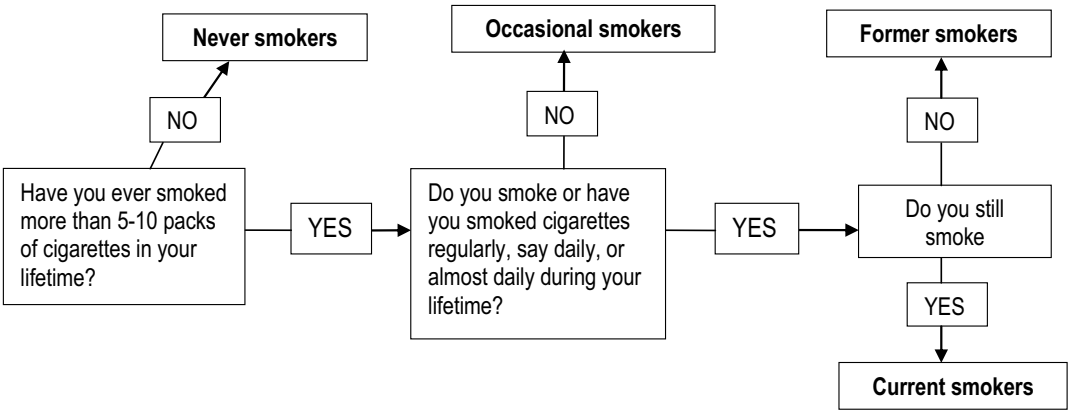

Figure 2. Definition of smoking status in the twin cohort questionnaires in 1975, 1981, and 1990.

More detailed smoking subgroups were formed according to the change in smoking status between 1975 and 1981. In Study I, only baseline light smokers were divided into subgroups, whereas in Studies II and IV all 1975-1981 participants were categorized according to change in their smoking patterns. See Statistical methods and Figure 3 for details. Formation of the smoking subgroups used in Studies II and IV is detailed in Appendix 1.

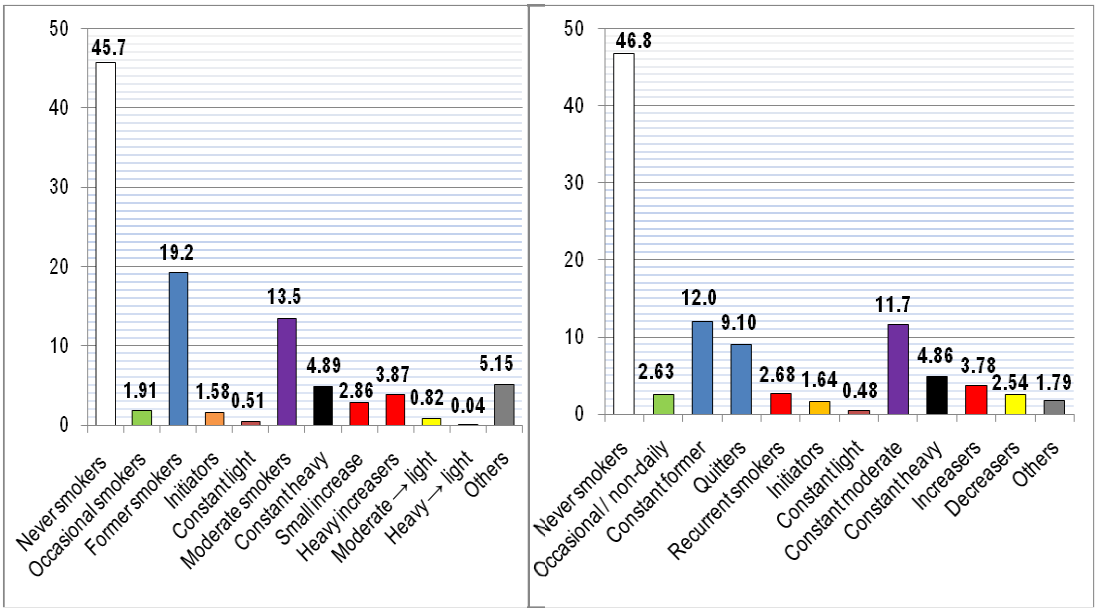

Figure 3. Smoking subgroup classification and proportions of different subgroups used in Study II (on the right) and in Study IV (on the left). Subgroups with similar smoking habits are presented with the same color. Detailed formation of the subgroups is presented in Appendix 1.

4.2.1.2. Questionnaires of the FITSA study

In the FITSA study, smoking status was assessed in the 2000 and 2003 questionnaires by asking: "Have you ever smoked more than 5-10 packs of cigarettes?" Negatively responding subjects were considered never smokers (n=374, 86% of the study population). Current smoking was also defined in the questionnaire, and none of those declaring themselves as never smokers reported current smoking.

4.2.2. Chronic bronchitis

Chronic bronchitis was assessed in the 1975 and 1981 questionnaires by asking whether subjects suffered from chronic cough and mucus production from the airways. Respiratory symptoms were monitored with the MRC series of questions (British Medical Research Council 1960), by asking: "Do you regularly for extended periods of time have a cough?", "How many months in a row do you cough per year?", "For how many months in a row do you bring up phlegm from your chest per year?". Those reporting suffering from the symptoms over at least three successive months a year were considered to have chronic bronchitis. Questions were asked identically in both surveys, and chronic bronchitis was dichotomized as 0 or 1. Chronic bronchitis was the main outcome in Study II, and it was used as a potential confounder in Study IV.

4.2.3. COPD

To define COPD, a record-linkage study was performed by combining the data of the 1975 and 1981 twin cohort surveys with the SII's nationwide medical registry data. Data of diagnoses entitling to special reimbursements (between 1981 and 2008) as well as data on purchased basically reimbursed medications (1995-2008) were obtained for those 21 609 subjects who participated in both 1975 and 1981. Because inhaled anticholinergics and combinations of inhaled anticholinergics and $beta_2$ agonists are generally not used to treat conditions other than COPD, purchase dates of such medications were monitored. These medications included oxitropium, ipratropium, and tiotropium, as well as combinations of ipratropium and salbutamol, and ipratropium and fenoterol. A similar definition of COPD has been used previously (Tynkkynen et al. 2009).

In this study, COPD was defined as a diagnosis of emphysema, chronic bronchitis, or COPD entitling to special reimbursement, or as regular usage of inhaled anticholinergics

preparations. Regular usage was considered to be at least two purchases at 99-day intervals. Subjects who moved abroad, were deceased, or had a special reimbursement for COPD, chronic bronchitis, or emphysema before the start of the follow-up were excluded from the analyses.

4.2.4. Lung function

Lung function of the FITSA study participants was assessed with spirometry measures in 2000 and 2003 by using an electronic spirometer (Medikro 202, Medikro Co., Kuopio, Finland). At baseline, 397 subjects provided acceptable spirometry performances, and of these, 339 were never smokers and were included in our study. At follow-up, 287 subjects, 252 of whom were never smokers, provided adequate performances.

Flow-volume spirometry, including all values except post-bronchodilator measures, was performed according to international guidelines (Miller et al. 2005b). Trained laboratory nurses made the measurements and guided the participants. The subjects were asked to inhale maximally and to exhale into a mouthpiece connected to a flow transducer. Slow and fast expiratory maneuvers were practiced before actual measurements. Spirometry was performed in a standing position, and subjects sat down after each performance. Maneuvers were performed until at least two were acceptable, and of these, the better one was recorded as the subject's result. Nose clips were used, and if the testee had dentures, spirometry was carried out without them. The spirometer was calibrated daily and was accurate to within 1%. Age- and height-adjusted reference values were derived from a national sample, recommended to be used in Finns (Viljanen 1982, Sovijärvi et al. 2006). In Study III, FEV1, FVC, and FEV1/FVC ratio measurements derived from both time-points were analyzed as continuous variables.

4.2.5. Potential confounders

A confounding variable is an extraneous factor that is statistically related both to the outcome and to the exposure. To control for such factors in this study, the final statistical models investigating the association between the exposure and the outcome were adjusted for multiple potential confounders. Known confounding factors from the literature were taken into consideration, if data were available. The effects of other potential confounders were tested by adding such variables in the statistical model. If a potential confounding variable

changed the observed risk estimates in the model, it was included in the final multivariate model.

4.2.5.1. Twin cohort variables

The 1975 and 1981 questionnaires were composed of an extensive battery of questions concerning health, health behavior, and sociodemographic factors. In Studies I, II, and IV, the variables age, sex, alcohol use, physical activity, and the age at smoking initiation were used as potential confounders. In addition, marital status, pipe and cigar smoking, inhalation pattern, coffee and tea drinking, life satisfaction, and stress of daily activities were adjusted for in Study I. Education was adjusted for in Studies I and IV. Asthma and pack-years were used in Studies II and IV, and allergic rhinitis and occupation in Study II. The variable 'duration of cessation' (among former smokers) was created and used in Studies II and IV.

Age was used as a continuous variable, and in Study I, was also categorized into four groups: ≤25, 26-30, 31-40, and >40 years. Alcohol use was regarded as 'heavy' if the participant reported having six or more drinks on one occasion at least monthly (Kaprio et al. 1987). Physical activity was categorized as sedentary, intermediate, or active based on frequency, duration, and intensity of physical activity (Kujala, Kaprio & Koskenvuo 2002). Daily coffee and tea drinking were assessed as continuous variables by the number of cups per day.

The variable 'pack-years' was calculated according to average daily cigarette consumption and age at regular smoking initiation. According to the age at smoking cessation, duration of cessation was categorized in Study II as >10, 2-10, and <2 years, and in Study IV as >10, 5-10, and <5 years. Current and former smokers were asked whether or not they inhaled smoke while smoking. Reporting having ever smoked at least 50 cigars or 75 cigarillos, or more than 3-5 packages of pipe tobacco, was considered as lifetime pipe/cigar smoking. Self-reported age at regular smoking initiation was used as a continuous variable.

Education was dichotomized as those with at least senior high school completed and those with lower education. Marital status was coded in the questionnaire as unmarried / married / remarried / living with a partner / divorced / widowed. In Study I, those who were single in 1975 were used as a reference group, those married or living with a partner formed another group, and those separated or widowed composed the third group. Occupation was coded as upper-level employee or large entrepreneur / lower-level employee or small entrepreneur / trained worker / untrained worker / farmer, fisher, or gardener / unknown.

Asthma and allergic rhinitis were screened by asking "Has your doctor ever told you that you have asthma/allergic rhinitis?" Life satisfaction was assessed by a four-item scale focusing on feelings of loneliness, hardness of life, happiness, and anhedonia (Koivumaa-Honkanen et al. 2004). Being tense and nervous, having stress in daily activities, being mentally and physically exhausted at the end of the day, and daily activities being extremely trying and stressful were reported by subjects on a scale from 1 to 4 (Reeder, Schrama & Dirken 1973), and the total score (4-16) was used to define stress of daily activities (Korkeila et al. 1998).

4.2.5.2. Variables of the FITSA study

The general questionnaire of the FITSA study included in both years 189 questions concerning sociodemographic factors and health behavior. In addition, a separate health questionnaire included over 200 specific questions concerning health. Pulmonary diseases, BMI, height, physical activity, and occupation were examined as possible covariates. Weight and height were measured in the laboratory, and BMI was calculated as a function of weight and height (kg/m^2).

Pulmonary morbidity was screened by asking whether one suffered from chronic cough or sputum production, or chronic bronchitis, chronic or seasonal asthma, emphysema, or other pulmonary diseases, such as tuberculosis, alveolitis, bronchitis, pneumothorax, pneumonia, embolus, sarcoidosis, pleural effusion, fibrosis, allergy, or chronic obstructive pulmonary disease. A dichotomous variable describing lung health was created, which was coded as 1 if the participant reported suffering from any of the above-mentioned disorders.

Leisure-time physical activity was scaled in the questionnaire as following: No leisure-time physical activity (0), light walking 1-2 (1) or several times (2) a week, moderate activity 1-2 (3) or several times (4) a week, vigorous activity (5), and competitive sports (6). Subjects in categories 0 and 1 were considered as sedentary, 2-3 as engaging in moderate activity, and 4-6 as engaging in high activity (Schroll 2003).

4.3. Statistical methods

4.3.1. Individual-based analyses

In Studies I, II, and IV, the twins were analyzed mostly as individuals. Statistical analyses were performed with Stata statistical software (versions 9-11), where the possible correlation

between twin individuals can be controlled for by using the cluster option and robust estimators of variance (StataCorp. 2009).

For Studies I, II, and IV, smoking subgroups taking into account changes that occurred in smoking patterns between 1975 and 1981 were formed. In Study I, future smoking patterns of the 1975 light smokers were analyzed, and three groups were formed according to their consumption in 1981 (increasers, quitters, continuous light smokers). In Study II, more detailed subgroups were formed, and categories with similar changes in smoking patterns were grouped together (Figure 3). In Study IV, the disease risks for the initial 36 smoking categories were first explored with regression models, and then the categories for which risk estimates could be set as equal in the regression model were combined (Figure 3).

The associations between possible confounding factors and outcomes were explored in Studies II and IV with logistic regressions, and in Study III with linear regression models before proceeding to genetic modeling. Correlations between confounders were also assessed; in Studies II and IV, some potential confounders (smoking initiation age, pack-years) were excluded from the final models because of their high correlation with smoking categories ($\geq$0.6).

Adjusted logistic regression models were used in Studies I, II, and IV, and odds ratios (ORs) with 95% confidence intervals (CIs) were computed for the outcomes. In Study I, logistic regressions were used to test the statistical significances of being a light versus a heavy smoker and to examine longitudinally the predictors for continuing light smoking versus increasing smoking or quitting during the follow-up. In Studies II and IV, the risks for incident chronic bronchitis and COPD were tested with logistic regressions among different smoking categories, also among subgroups taking into account the changes in smoking patterns between 1975 and 1981. Never smokers were used as the reference group (OR=1.00).

In Study II, further analyses were performed with random-effects logistic regression models after reshaping the data as longitudinal panel data, consisting of repeated measures of smoking and chronic bronchitis. The random-effects model analyzed the risk of chronic bronchitis associated with smoking patterns at all measurement points simultaneously by assuming that subject's smoking status at certain measurement point is independent of other measurements (Diggle et al. 2002, Twisk 2003).

Survival analyses were used in Study IV when exploring the risk of developing COPD during follow-up. The risks were estimated according to smoking status in 1981, as well as according to changes in smoking patterns between 1975 and 1981. Because follow-up times

were different for special reimbursement eligibilities (1981-2008) and inhaled anticholinergic purchases (1995-2008), analyses were performed separately for both outcomes (study design presented in Figure 4). The follow-up for individuals ended on the date of granting a special reimbursement / initiation of regular medication use, or migration from Finland, death, or end of the follow-up. Hazard ratios (HRs) with 95% CIs for COPD development were calculated with an adjusted Cox proportional hazards regression model (Cox & Oakes 1984). Nonparametric Nelson-Aalen estimators were used when illustrating the cumulative hazard estimates for COPD for 1981 smoking groups.

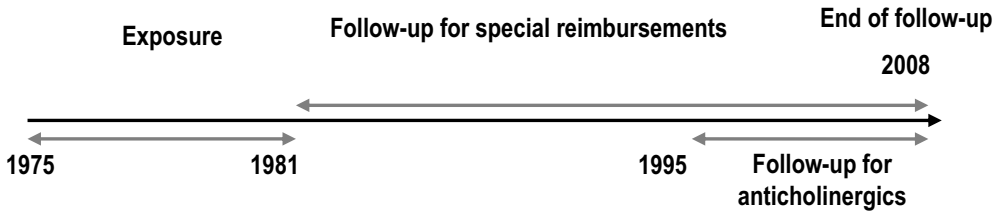

Figure 4. Design of the Study IV. Smoking was measured in 1975 and 1981.The follow-up for special reimbursement eligilibilities started in 1981, and the follow-up for anticholinergic medication in 1995. Both follow-ups ended in 2008.

4.3.2. Models based on twin pairs

4.3.2.1. Discordant twin pair analyses, Studies II and IV

Because MZ twins share 100% of their genes, and DZ twins share on average 50% of their segregating genes, greater resemblance between twins of a MZ pair is considered to be due to genetic influences. Accordingly, a disease affecting only one twin of a MZ pair must be caused by unique environmental factors. Same-sex MZ twin pairs discordant for an outcome phenotype are excellent for case-control studies since they are perfectly matched for genotype, sex, age and shared environmental influences (Boomsma, Busjahn & Peltonen 2002). Causal relationships between outcome phenotypes and associated risk factors can also be tested with discordant twin pairs. If the association observed in individual-based analysis remains significant in discordant MZ twin pairs, genetic and shared environmental factors affecting this association can be ruled out.

In Study II, twin pairs discordant for smoking status in 1975-1981 and for the incidence of chronic bronchitis in 1990 were analyzed (n=15 pairs). The twin with chronic bronchitis was the case, and the healthy co-twin was the control. Twins were considered discordant for smoking if one twin was a never smoker, and the co-twin a persistent or recurrent smoker. The ORs were calculated by the ratio of the number of case-control pairs in which the case smokes and the control does not smoke to the number of pairs in which the case does not smoke while the control smokes. The significance of the association within such pairs was tested with McNemar's test (Thomas 2004).

In Study IV, conditional logistic regression analyses were conducted for MZ and DZ twin pairs discordant for COPD (n=325; 213 DZ, 81 MZ pairs). The risk estimates related to smoking patterns were assumed to remain similar to those found in the whole sample, which would support a causal association between smoking and COPD, independent of genetic factors.

4.3.2.2. Quantitative genetic modeling, Study III

All analyses in Study III, except the initial models testing confounders, were based on the twinship of the study subjects. Correlations were calculated with Stata, whereas uni- and multivariate structural equation modeling (SEM) was implemented in Mx software and based on standard Mx scripts obtained from the GenomEUtwin Mx website (http://www.psy.vu.nl/mxbib/).

The classical twin design is based on the assumption that the phenotypic variance can be decomposed into additive genetic (A), dominant genetic (D) or shared environmental (C), and nonshared environmental (E) factors. The expected correlations for variance components A, D, and C between the twins of a MZ pair are 1.0, while for DZ pairs, the correlations are 0.5 for A, 0.25 for D, and 1.0 for C. E effects are, by definition, uncorrelated in both MZ and DZ twins. E also contains random effects and is therefore always included in the models. Further, correct estimation of genetic and environmental components requires that no GxE interaction, gene-environment (GE) correlation, or assortative mating exists. Estimation of GxE interaction and GE correlation requires special models. Also an assumption of equal environmental effects for MZ and DZ twins is required for the reliable estimation of variance components (Posthuma et al. 2003).

In Study III, intra-class correlation coefficients with 95% CIs were first computed for FEV1, FVC, and FEV1/FVC in MZ and DZ twin pairs to estimate the within-pair similarity.

Greater similarity between MZ than DZ pairs is suggestive of a genetic influence on the trait. Then, for each lung function parameter, cross-twin, cross-time correlations were computed for MZ and DZ pairs. A correlation between baseline measures in one twin and follow-up measures in the co-twin gives evidence whether shared genetic influences explain the covariance in the trait over time.

By SEM, the variance in the observed phenotype can be decomposed into A, E, and either C or D components simultaneously. The mathematical models may also be represented by using path diagrams (see Figure 5) (Evans, Gillespie & Martin 2002). The basic assumptions of genetic modeling (equal means and variances for MZ and DZ twins) are tested by comparing the genetic models with saturated models, which make no such assumptions. If the fit of genetic models to the data is not significantly worse than that of saturated models, it means that these assumptions are fulfilled. SEM is based on comparing simplified models with the model in which they are nested. Starting with a full ACE or ADE model, variance components are dropped one at a time, and the fit of simplified models (AE, CE, E) to the data is tested. The aim is to obtain the most parsimonious and best-fitting model to explain the observed pattern of similarity in MZ and DZ pairs (Boomsma, Busjahn & Peltonen 2002). Likelihood ratio tests and Akaike's Information Criterion (AIC) are used to compare alternative models, whereas χ^2- and p-values are applied to compare nested submodels with the more saturated ones (Neale & Maes 2006). In Study III, univariate models were used to test the associations of covariates with lung function phenotypes (see path diagram in Figure 5 as an example of a univariate ACE model).

The univariate model can be extended to a multivariate model when more than one measurement per subject is available. Such models give evidence of whether genetic effects are shared by repeated measurements or whether they are specific to a certain measurement point. Since spirometry was performed at two time-points, a bivariate Cholesky decomposition (Posthuma et al. 2003) (Figure 6) was used to explore the data. In such an ACE or ADE model, effects A_1, C_1, D_1, and E_1 are common to baseline and follow-up, while A_2, C_2, D_2, and E_2 load only on the later measurement point. Heritability estimate of the phenotype is the fraction of genetic variance from the total variance. Variances are derived by squaring the path (standard) coefficients *x*, *y,* and *z* of the latent variables A, D or C, and E. The phenotypic variance for the trait thus equals $x^2 + y^2 + z^2$ (Boomsma, Busjahn & Peltonen 2002).

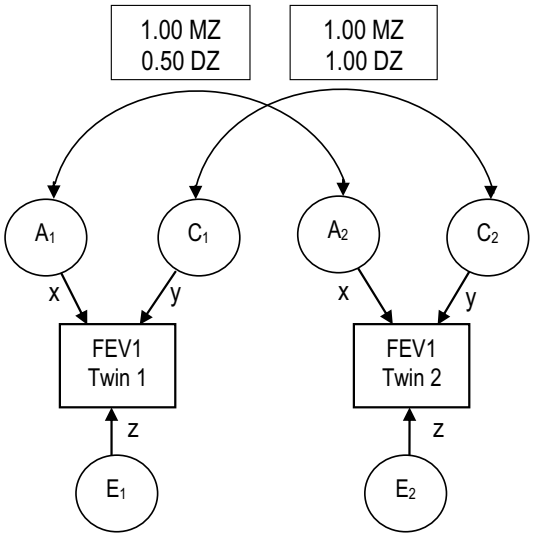

Figure 5. Univariate twin model illustrating the similarity of a trait (FEV1) in a MZ or DZ twin pair. The variance in the trait is composed of latent variables A, C, E, and x, y, and z represent the respective path coefficients on the trait. The variance in the phenotype also equals $x^2+y^2+z^2$. The path coefficients can be regarded as standardized regression coefficients. An ADE model would include D effects instead of C; C and D effects cannot be estimated simultaneously as they are confounded.

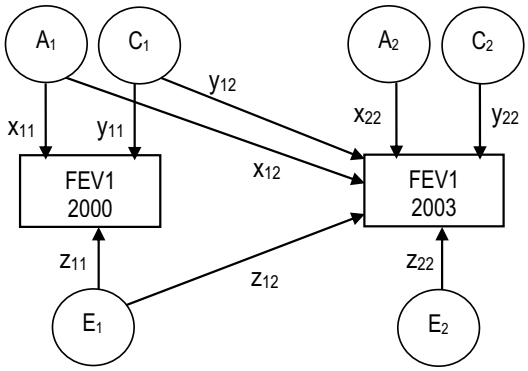

Figure 6. A bivariate ACE Cholesky model illustrating genetic and environmental effects loading on two measurement points of FEV1 in one twin. Effects A_1, C_1, and E_1 are common to both measurement points, whereas A_2, C_2, and E_2 load only on the later measurement. The alternative model would include effect D instead of C.

5. Results

5.1. Various smoking patterns

5.1.1. Prevalence of different smoking patterns in 1975-1981

Proportions of different smoking status groups for the 21 609 subjects who participated both in 1975 and 1981 are shown in two columns in Figure 1. In 1975, 31.5% of subjects were current smokers, and, according to daily smoking amount, were grouped as light (8.1%), moderate (67%), and heavy smokers (25%); representing 2.5%, 21%, and 8% of the total population, respectively (Figure 7).

By 1981, the proportion of former smokers in the sample had increased (from 16.4% to 21.2%), that of moderate smokers had decreased (from 21% to 16.7%), and that of other smoking groups had remained rather stable (Figure 7). The majority of former, moderate, and heavy smokers reported a similar status also in 1981 (73%, 56%, and 61%) (not shown in Figure 7). However, light smoking showed a less consistent smoking pattern at follow-up. The 1981 smoking patterns of the baseline (1975) light smokers are described in Figure 7, where almost half had quit smoking, about one-fifth had increased to heavy or moderate smoking, and almost one-third had continued light smoking.

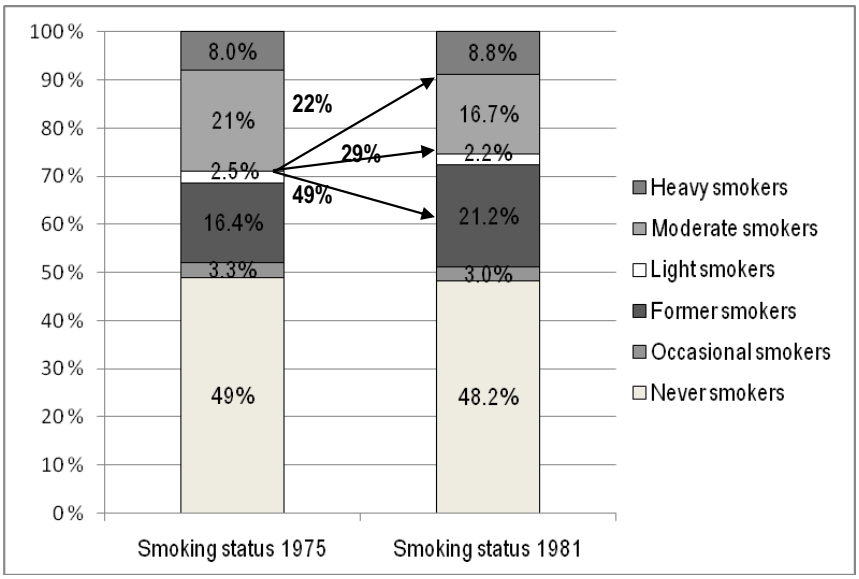

Figure 7. Proportions of different smoking status groups among the study population in 1975 and 1981, and the distribution of baseline light smokers according to smoking status in 1981 (increasers, 22%; continuous light smokers, 29%; quitters, 49%). Note: The percentages have been rounded and do not sum exactly 100%.

When taking into account smoking status at both time-points and examining the smoking subgroups describing change in smoking in 1975-1981, constant never smokers comprised half of the study population, whereas constant moderate smokers, constant former smokers, and quitters comprised about one-tenth each. Other smoking subgroups, e.g. decreasers, increasers, and relapsed former smokers, were relatively smaller (see Figure 3 in Methods for details).

5.1.2. Consistency of light smoking

The 1981 smoking patterns of the baseline (1975) light smokers are presented in Figure 7. About half of the 1975 light smokers attended again in 1990, when 53% reported being former, 33% moderate / heavy, and 14% light smokers. At a population level, the share of light smokers remained quite stable: 8.0% and 8.2% of current smokers reported consuming 1-4 CPD in 1981 and 1990. Only 5.9% of the baseline light smokers reported the same consumption throughout the 15 years; smoking reducers, initiators, and relapsed former smokers formed new members in the group of light smokers (see Figure 8).

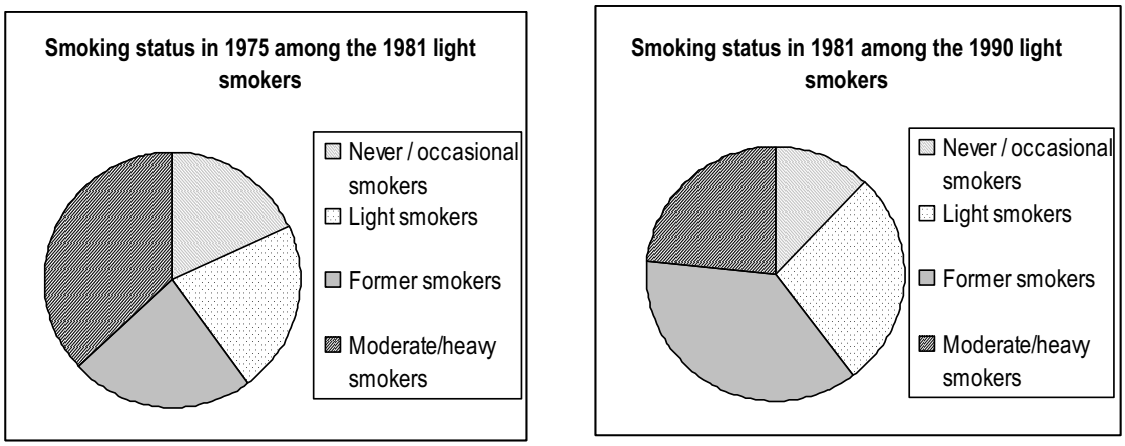

Figure 8. Previous smoking status of those who reported being light smokers in 1981 and 1990.

5.1.3. Characteristics of light smokers

Characteristics of the 1975 light smokers were compared with heavy smokers by using age- and sex-adjusted logistic regressions. The likelihood of light versus heavy smoking was elevated among women, physically active and better educated subjects, tea drinkers, those

reporting older age at smoking initiation, and those reporting less stress. The likelihood of light smoking was lower among older smokers, lifetime pipe/cigar smokers, coffee drinkers, those ever married or living with a partner, and those reporting inhalation of tobacco smoke, heavy alcohol use, or dissatisfaction with life.

Baseline light smokers were divided into groups according to their smoking patterns in 1981 (increased, quit, or continued light smoking). Associations between their other characteristics at baseline and 1981 smoking patterns were studied. Baseline age of 26-30 or ≥40 years predicted continued light smoking versus quitting, whereas being single in 1975 but living with a partner in 1981 predicted change in smoking. Higher education at baseline predicted continued light smoking versus increasing smoking, whereas heavy alcohol use at baseline predicted the opposite pattern.

5.2. Heritability of lung function

5.2.1. Lung function and characteristics of the FITSA participants

Among never-smoking participants, the mean age at baseline was 68.7 years and the lifetime pulmonary disease prevalence was 20%. Over half of these female subjects reporting pulmonary diseases suffered from asthma (59% at baseline, 64% at follow-up), the second most common lung disease being chronic bronchitis, respectively (14% and 11%). Mean spirometry values of those never smokers who attended measurements at both time-points are presented in Table 1.

Table 1. Mean spirometry values of the never-smoking MZ and DZ participants of the FITSA study.

	FEV1 (MZ)	FEV1 (DZ)	FVC (MZ)	FVC (DZ)	FEV1/FVC (MZ)	FEV1/FVC (DZ)
Mean value (liters or %) with (SD) in 2000	2.29 (0.47)	2.18 (0.49)	2.91 (0.57)	2.76 (0.61)	73.22 (8.23)	75.44 (9.40)
Mean value (liters or %) with (SD) in 2003	2.22 (0.46)	2.14 (0.44)	2.77 (0.58)	2.70 (0.56)	77.91 (6.52)	76.90 (7.60)

5.2.2. *Lung function correlations*

The ICCs (see Table 1 in Study III) were greater for MZ than DZ pairs, suggesting a genetic background in the observed variance in FEV1, FVC, and the FEV1/FVC ratio. Only the ICCs for FEV1/FVC at follow-up did not differ between MZ and DZ pairs.

Cross-twin, cross-time correlations for FVC are presented in Table 2 (see results for FEV1 in Table 2, Study III). The correlation of FVC 2000 of twin 1 and FVC 2003 of twin 2 was 0.52 among MZ pairs and 0.40 among DZ pairs, suggesting that the same genes would affect that phenotype at both measurement points.

Table 2. Correlations between FVC in 2000 and in 2003 in MZ and DZ never-smoking twin pairs. Correlations for MZ pairs are in bold; those for DZ pairs in italics.

	Twin 1		Twin 2	
	FVC in 2000	FVC in 2003	FVC in 2000	FVC in 2003
Twin 1 FVC in 2000	-	**0.79**	**0.71**	**0.52**
FVC in 2003	*0.75*	-	**0.51**	**0.44**
Twin 2 FVC in 2000	*0.25*	*0.18*	-	**0.81**
FVC in 2003	*0.40*	*0.36*	*0.70*	-

5.2.3. *Genetic and environmental influences on FEV1, FVC, and FEV1/FVC*

Covariates for bivariate modeling were chosen according to the results of the linear regression models and univariate genetic models. The model for FEV1 was adjusted for age, BMI, physical activity, and lung health, explaining about 20% of the variance at both time-points. The model for FVC was adjusted for age, BMI, and physical activity, and the model for FEV1/FVC for age, height, lung health, and physical activity. Covariates were chosen from the best-fitting genetic model after comparing alternative models with different covariate combinations. Covariates explained 15% of the variance in FVC, and about 8% of the variance in FEV1/FVC. Full Cholesky models were reduced to obtain the best-fitting models, which were AE models for each variable.

The proportion of variance in FEV1 explained by each latent factor is illustrated in Figure 9. The genetic correlation approached unity, indicating that genetic factors affecting FEV1 were common at both measurements and no genetic innovations appeared. Instead, new environmental factors appeared at follow-up. The environmental correlation between baseline and follow-up was 0.69 (95% CI 0.56-0.80). The path coefficients with 95% Cls are illustrated in Figure 2, Study III.

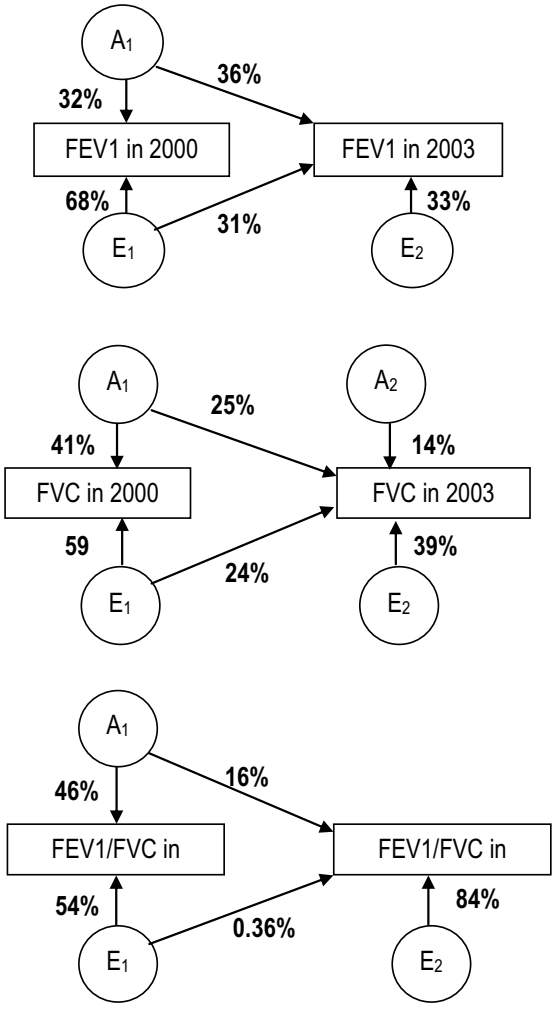

Figure 9. The most parsimonious Cholesky decomposition models for FEV1, FVC, and the FEV1/FVC ratio at baseline in 2000 and at follow-up in 2003. The model consists of additive genetic effect A_1 and nonshared environmental effect E_1 in common for both measurement points. Each phenotype measured in 2003 has its own nonshared environmental effect E_2. In addition, FVC measured in 2003 has its own additive genetic effect A_2. The percentages represent the proportion of variance explained by each latent factor at each time-point and were derived by squaring the path coefficients x, y, and z.

In the final Cholesky model for FVC (Figure 9), both new genetic and new environmental effects appeared at follow-up. The genetic correlation between baseline and follow-up was 0.80 (95% CI 0.44-1.00). Nonshared environmental effects explained 59% (95% CI 37-87) of the variance at baseline and 63% (95% CI 43-88) at follow-up. The environmental correlation between baseline and follow-up was 0.62 (95% CI 0.41-0.77). The path coefficients with 95% Cls are illustrated in Figure 3, Study III.

For the FEV1/FVC ratio (Figure 9), the proportion of variance explained by genetic effects diminished substantially by the follow-up; however, the genetic correlation approached unity. Environmental correlation was 0.07 (95% CI -0.18-0.37). The path coefficients with 95% CIs are illustrated in Figure 4, Study III.

5.3. Chronic bronchitis

5.3.1. Incidence of chronic bronchitis and its association with smoking patterns

The incidence of chronic bronchitis was studied in 1981 according to 1975 smoking status, and by excluding those with chronic bronchitis in 1975. Among the whole sample, the incidence was 1.7%, and among 1975 current smokers 3.0%. The risk increased linearly, about 1.5-fold, by each amount category of daily cigarettes among 1975 smokers (OR 1.54, 95% CI 1.38-1.73). Despite this linear trend, when examining 1975 smokers as categories, only moderate (OR 3.03, 95% CI 2.26-4.06) and heavy smokers (OR 6.75, 95% CI 4.88-9.35) had an increased risk for incident chronic bronchitis relative to never smokers.

Further, when examining the risk of chronic bronchitis in 1981 among the smoking subgroups describing the change in smoking in 1975-1981, in addition to constant moderate (OR 2.73, 95% CI 1.89-3.94) and heavy (OR 10.04, 95% CI 7.03-14.35) smokers, also smoking increasers (OR 8.10, 95% CI 5.42-12.10), decreasers (OR 3.78, 95% CI 2.16-6.62), and recurrent smokers (OR 2.54, 95% CI 1.31-4.93) were at increased risk for chronic bronchitis (see Table 1 and Figure 1, Study II).

To take full advantage of the repeated measurements of smoking status and chronic bronchitis between 1975 and 1990, we reshaped the data as panel data and constructed random-effects logistic regression models. Panel analyses according to smoking status showed that the risk of chronic bronchitis was elevated also among former smokers, as well as among the daily light, moderate, and heavy smokers, relative to never smokers (see Figure

10). When dividing former smokers into subgroups according to the duration of abstinence, those abstinent for over ten years had a significantly lower risk compared to those abstinent for less than two years.

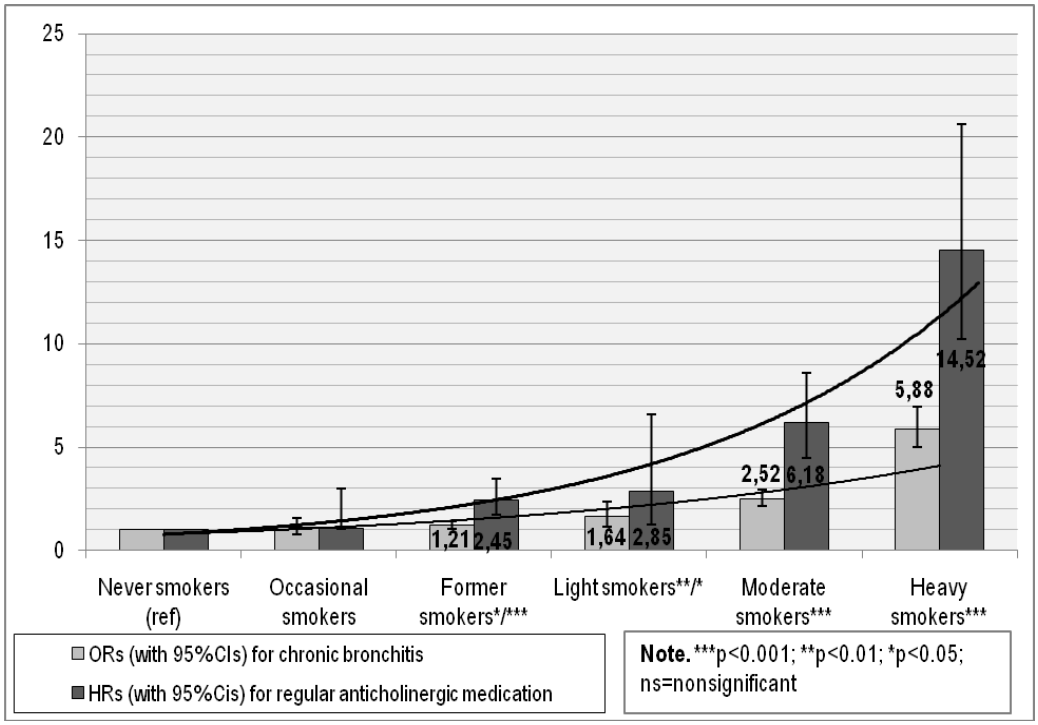

Figure 10. ORs of chronic bronchitis (1981) as well as HRs of regular anticholinergic medication (1995-2008) with 95% CIs according to smoking categories. ORs are based on random-effects model analysis carried out on the panel data, and HRs are based on the Cox proportional hazards regression model for regular anticholinergic medication. Never smokers were used as a reference category. Continuous lines represent the results of trend test (thick line for regular anticholinergic medication, thin line for chronic bronchitis).
For former smokers, p≤0.05 for chronic bronchitis and p≤0.001 for medication. For light smokers, p≤0.01 for chronic bronchitis and p≤0.05 for medication. Occasional smokers' risks were nonsignificant.

5.3.2. Discordant twin pair analyses

Among twins who participated in all three surveys, there were 112 pairs discordant for incident chronic bronchitis in 1990. Among them, 15 twin pairs were discordant also for 1975-1981 smoking status. In 14 pairs, the smoker had developed chronic bronchitis, whereas in one pair, the never-smoker was affected. With conditional logistic regression models, the OR for chronic bronchitis was 14.95 (95% CI 1.8-107) in smoking twins as compared with their never-smoking co-twins. When the analysis was restricted to DZ pairs (n=12), the OR

was 11 (95% CI 1.4-85). In three MZ pairs, there were no cases where the never-smoker twin had chronic bronchitis, and the analysis could not be conducted.

5.4. Chronic obstructive pulmonary disease

5.4.1. Incidence of COPD and its association with smoking patterns

The cumulative incidence of COPD special reimbursement eligibility was 0.54% among all participants during a 27-year follow-up. The incidence of regular anticholinergic use was 2.15% in the whole sample and 3.86% in ever smokers during a 13-year follow-up. Incidence rates among the groups describing change in smoking are presented in Figure 11.

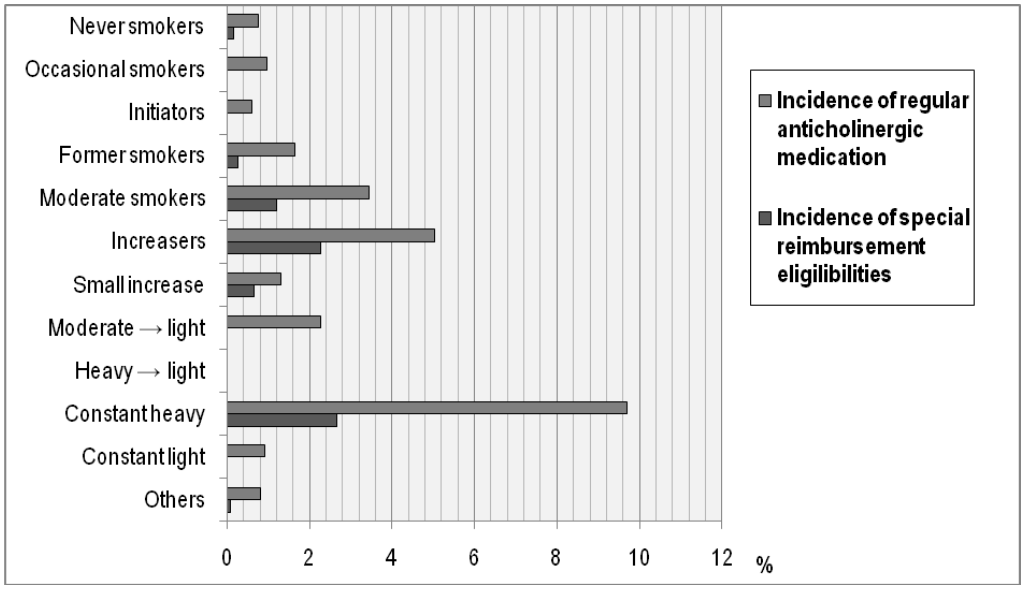

Figure 11. Cumulative incidence of COPD, measured as special reimbursement eligibility and regular anticholinergic use, according to smoking patterns describing change in smoking in 1975-1981.

The risk of COPD, measured as regular anticholinergic medication or special reimbursement eligibility, was first studied according to smoking categories in 1975 and 1981. Logistic regression models showed that the disease risk was significantly increased among 1975 and 1981 former, moderate, and heavy smokers relative to never smokers. Survival analyses, conducted separately for both outcomes, revealed that all 1981 daily smokers, also light smokers, had elevated risks for regular anticholinergic medication relative to never smokers (see Figure 10). By contrast, significantly elevated HRs for special

reimbursement eligibility were observed only among moderate and heavy smokers (see Study IV for details).

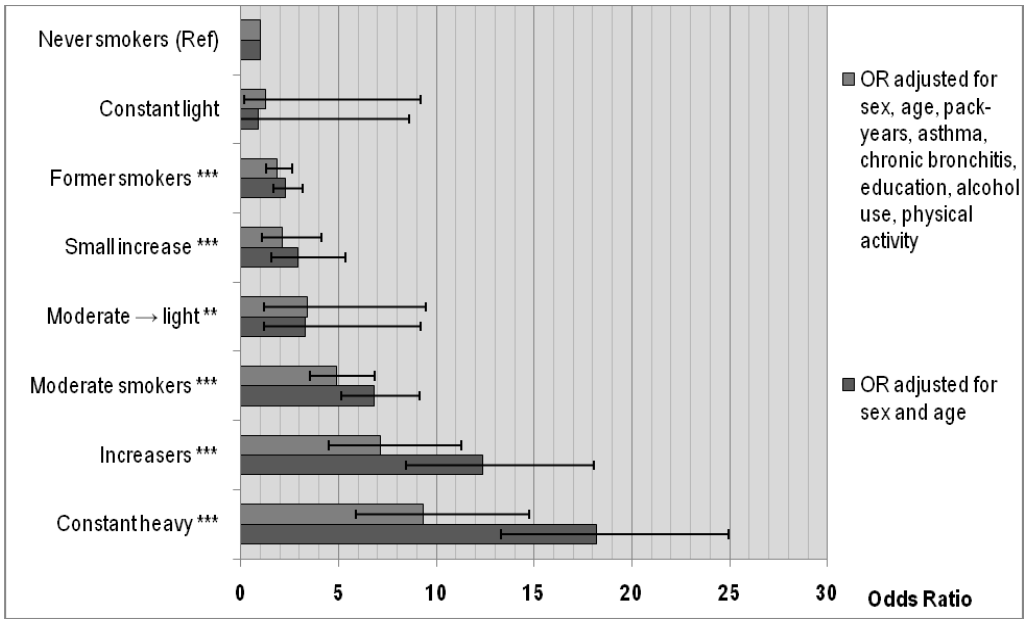

Figure 12. Risk for COPD development during follow-up by smoking patterns taking into account change of smoking between 1975 and 1981. ***p≤0.001, **p≤0.01. For the group "moderate → light", p-value from the multivariate-adjusted model ≤0.01, p-values from the age- and sex-adjusted model ≤0.05. For the group " small increase" the p-value from the age- and sex-adjusted model was ≤0.001, the p-value from the multivariate-adjusted model was ≤0.05. Heavy→light smokers, initiators, and occasional smokers not shown.

When taking into account the change in smoking between 1975 and 1981, an increased risk for COPD was found for all daily smokers, other than constant light smokers. Results of the logistic regression models suggest that those who increased to heavy smoking, reduced from moderate to light smoking, increased from light to moderate smoking, or relapsed back to light or moderate smoking after quitting were at increased disease risk (see Figure 12). The main results were replicated in survival analyses, where the HR for regular anticholinergic medication was similar among those who reduced from moderate to light smoking as among smoking increasers and constant moderate smokers (see summary of the results of survival analyses in Table 3). For details, see Figure 2 and Table III in Study IV.

The risk of having ever used anticholinergic medication was further studied among subgroups describing change in smoking. Former, constant light, moderate, and constant heavy smokers as well as smoking increasers had purchased combination preparations of

anticholinergics and beta agonists significantly more often than never smokers. For pure anticholinergics, elevated risks were observed for the same smoking groups, excluding constant light smokers. A summary of the risks of inhaled medication use according to change in smoking status is presented in Table 3. Detailed results are reported in Study IV.

Table 3. Summary of the results of COPD risk according to change in smoking patterns. Results are based on logistic regression analyses of anticholinergic medication and survival analyses of anticholinergic medication and special reimbursement eligibilities. Categories describing change in smoking in 1975-1981 are presented on the left. Occasional smokers, initiators, heavy→light smokers not shown. ↑ significantly increased risk ($p<0.05$), ↔ nonsignificant risk relative to constant never smokers. Constant light smokers and moderate→light smokers were omitted from the survival analyses of special reimbursements because there were no affected cases among these groups.

	Combination preparations (logistic regression)	Pure anticholinergics (logistic regression)	Regular anticholinergic medication (survival analysis)	Special reimbursement eligibility (survival analysis)
Former smokers	↑	↑	↑	↔
Constant light smokers	↑	↔	↔	Omitted
Moderate→light	↔	↔	↑	Omitted
Small increase	↔	↔	↔	↑
Constant moderate	↑	↑	↑	↑
Constant heavy	↑	↑	↑	↑
Increasers	↑	↑	↑	↑

5.4.2. Discordant twin pair analyses

Conditional logistic regression analyses were conducted for twin pairs discordant for COPD. The risk estimates related to smoking patterns were similar to those seen when analyzing the data as individuals; risk of COPD was significantly elevated among smoking increasers (OR 6.15, $p=0.004$) and moderate (OR 8.98, $p\leq0.001$) or heavy smokers (OR 10.2, $p\leq0.001$). When the analyses were conducted separately for MZ and DZ pairs, the results in DZ pairs remained significant (moderate smokers: OR 10.8, $p\leq0.001$; increasers: OR 7.71, $p=0.007$; heavy smokers: OR 9.36, $p=0.004$). Although not reaching statistical significance, the risk estimates remained high also for MZ pairs; the ORs were 7.69 for moderate and 24.6 for heavy smokers, whereas no COPD cases among increasers were observed.

6. Discussion

6.1. Summary of aims and results

The first aim was to study the prevalence and consistency of different smoking patterns in a Finnish population by focusing on the subgroup of daily light smokers, and to examine the characteristics of Finnish light smokers. In a representative Finnish population sample, daily light smokers were found to more likely be females, well-educated, physically active, and exhibiting other healthy lifestyle characteristics than heavy smokers. Daily light smoking was a temporary pattern for most individuals, whereas at the population level, the proportion of light smokers remained constant.

The second aim was to explore the heritability of lung function by using spirometry results of never-smoking twins from two time-points. Among elderly never-smoking females, genetic effects explained about one-third of the observed variance in FEV1 and FCV, and 16-46% of the variance in the FEV1/FVC ratio. The genes influencing FEV1 and FEV1/FVC were the same at different time-points, whereas for FVC, new genes explaining 14% of the variance were observed at follow-up. Environmental factors other than smoking were found to be important determinants of lung function.

The third aim was to estimate the incidence and risk of chronic bronchitis according to various smoking patterns by taking into account changes in smoking over time. The risk of incident chronic bronchitis was increased among constant heavy and moderate smokers, among those who increased or decreased their smoking during follow-up, and among those who relapsed back to regular smoking after quitting. Further analyses with panel data showed that the risk of chronic bronchitis was also increased among light and former smokers when the change in smoking status was not taken into account.

The fourth aim was to estimate the incidence and risk of COPD over a 27-year follow-up according to changes in smoking patterns that occurred before the beginning of the follow-up. Constant heavy and moderate smokers, smoking increasers, those who decreased from moderate to light smoking, or those who relapsed back to moderate or light smoking after quitting were at increased risk for COPD. Light smoking measured at one time-point was also associated with an increased COPD risk, and constant light smoking was associated with an increased use of anticholinergic medication.

6.2. *Main results and comparison with previous studies*

6.2.1. *Light smoking*

By comparing the characteristics of light smokers with those of heavy smokers, the likelihood of light smoking was found to be increased among women, subjects with higher education, physically active subjects, tea drinkers, and subjects reporting less stress and older age at smoking initiation. Use of other tobacco products, heavy alcohol and coffee consumption, and being married or living with a partner, by contrast, were more likely present in heavy smokers. Among current smokers, the proportion of light smokers remained constantly at 8%, although on an individual level this pattern was rarely consistent. Baseline age of 26-30 years or over 40 years predicted continuous light smoking versus quitting. Change in marital status predicted change in light smoking pattern, and higher education predicted continuous light smoking versus increasing smoking. Baseline heavy alcohol use predicted increasing smoking versus continuous light smoking.

The observed prevalence of light smoking is in line with international studies from the 1990s (Rosengren, Wilhelmsen & Wedel 1992, Owen et al. 1995, Zhu et al. 2003). More recent studies have, however, shown that the proportion of light smokers is increasing in countries with decreasing overall smoking prevalence (Pierce, White & Messer 2009, Shiffman 2009). In 2009, the proportion of light smokers among American smokers was estimated to be 15% (Shiffman 2009). One limitation of this study is that the screening of smoking patterns took place over 30 years ago, and the results are not applicable to the 21st century Finnish population. The subgroup of light smokers in the population may have changed after the participants attended the study. Interestingly, the proportion of occasional smokers in the Finnish population has not changed significantly since the 1970s (Helakorpi et al. 1999, Luoto, Uutela & Puska 2000, Helakorpi, Laitalainen & Uutela 2010), although the prevalence of occasional smoking is increasing in many other countries (Shiffman 2009).

Light smokers exhibited healthier lifestyle and mental health characteristics than heavy smokers. Those who increased from light to heavy smoking were already at baseline more stressed and dissatisfied than other light smokers. The associations between smoking and alcohol and coffee consumption are well-known (Carmody et al. 1985, John et al. 2003), and heavy smoking has previously also been shown to be associated with stress (Nielsen et al. 2008). Higher education and physical activity among light smokers suggest that they are more aware of the health effects of smoking than heavy smokers and possibly aim at harm

reduction. The higher prevalence of light smoking among unmarried subjects has also been previously demonstrated (Hyland et al. 2005). As quitting rates are higher among married and better-educated subjects (Broms et al. 2004), it seems logical that moving in with a partner predicted change in light smoking also in this sample. In conclusion, earlier research on light smokers and their characteristics is in line with the results of Study I (Okuyemi et al. 2002, Hyland et al. 2005, Pierce, White & Messer 2009). The results support the view that the population of light smokers in Finland is very similar to that in other Western countries.

Reporting inhalation was more common among heavy smokers than light smokers. Although inhalation was crudely dichotomized in the questionnaire, and no biochemical validation of nicotine intake was used, this result suggests that light smokers are not be as nicotine-dependent as heavy smokers. Earlier studies, particularly by Dr. Saul Shiffman, have examined and discussed this issue extensively (Shiffman & Paty 2006).

6.2.2. Lung function

Heritability of lung function was estimated in a sample of elderly never-smoking women. Previous studies using twin modeling have reported slightly higher heritability estimates (McClearn et al. 1994, Palmer et al. 2001, Hallberg et al. 2010). The heritability of FEV1/FVC was found to be 46% at baseline, which is in line with previously reported (Wilk et al. 2000). At follow-up, however, the estimated heritability of FEV1/FVC was only 16%. This marked change during only a three-year follow-up may be explained by a concomitant increase in time-specific environmental effects, such as disease processes or accidents.

Results of the lung function heritability show that environmental factors other than smoking are important determinants of pulmonary function. In this study, all environmental effects were found to be nonshared, whereas earlier studies have reported that shared environmental effects also explain a small proportion of the variance in FEV1 and FVC (Palmer et al. 2001, Hallberg et al. 2010). The environmental factors seem to be time-specific; at follow-up, more than half of the environmental factors loading on FEV1 and FVC and all of those loading on FEV1/FVC were novel. Examples of environmental effects affecting lung function at both time-points are socioeconomic status, occupational exposure, and passive smoking. Disease processes, accidents, and life events, in turn, are examples of specific environmental factors during the follow-up. Disease processes affect nutritional status, physical activity level, and muscle strength, all of which have an impact on respiratory function. Life events, such as losses and depression, may also affect functional capacity.

Sarcopenia inevitably occurs in old people, decreasing the capacity of the thorax. The results emphasize the importance of physical activity, nutrition, rehabilitation, and interventions also in older age since environmental factors other than smoking can substantially contribute to lung function. Poor lung function, in turn, increases the risk for cardiovascular diseases, metabolic syndrome, and overall mortality (Sin, Wu & Man 2005, Kohansal et al. 2009, Steele et al. 2009).

Additive genetic effects explained one-third of the variation in FEV1 and FVC at both baseline and follow-up. The genetic influences loading on FEV1 and FVC have also been earlier reported to consist of additive genetic effects (Palmer et al. 2001, Zhai et al. 2007, Hallberg et al. 2010). For FEV1/FVC, the heritability estimate decreased substantially during follow-up. For FVC at follow-up, new genetic effects emerged and explained 14% of the variance, whereas for FEV1 and FEV1/FVC the genetic effects remained the same as at baseline. The results are in line with the current view that multiple genes contribute to lung function. Environmental effects seem to easily displace the influence of genes on FEV1/FVC since the observed variance in FEV1/FVC at follow-up was explained to a great extent by environmental factors.

The decomposition of observed lung function variance into time-specific genetic and environmental effects has not been performed earlier. Longitudinal results on lung function heritability exist only for the decline rates of FEV1, FVC, and their ratio (Gottlieb et al. 2001). One previous study estimated the heritability of FEV1 among never smokers; it was found to be about half of that observed in current smokers (Zhai et al. 2007). Studies reporting higher heritability estimates for FEV1 and FVC or VC have included both smokers and nonsmokers, and results have been based on single spirometry measurements (Palmer et al. 2001, Hallberg et al. 2010).

One limitation of this study is that the results are applicable only to elderly never-smoking Caucasian females; thus, no conclusions can be made regarding men or young women. However, as the prevalence of ever-smoking is much higher in elderly men than in women, excluding the effect of smoking would be more complicated. Few studies have examined the gender differences of lung function heritability, and their results are controversial (McClearn et al. 1994, Hallberg et al. 2010). No gender differences have been reported in GWAs investigating variants associated with lung function.

6.2.3. Chronic bronchitis

This study revealed that the risk of chronic bronchitis increases almost linearly with the daily amount of cigarettes. The six-year incidence of chronic bronchitis varied between 1.9% and 5.2% among smoker groups with elevated risks. Reduction from moderate to light smoking was associated with an elevated disease risk, showing that reduction does not protect from chronic bronchitis. By contrast, no elevated risk of chronic bronchitis was observed in quitters. The results support the view that smoking cessation may improve pulmonary symptoms, whereas smoking reduction does not offer any harm reduction. However, panel analyses studying the risk of chronic bronchitis according to smoking status found increased disease risks also in light and former smokers. This finding may reflect increased symptoms among those former smokers who had been abstinent for only a short period or who relapsed during the follow-up. Similarly, those light smokers who had previously smoked more or increased their smoking during follow-up were likely to exhibit an increased chronic bronchitis risk.

Earlier incidence studies on chronic bronchitis according to different smoking patterns are few. A Finnish cohort study found the lifetime incidence of chronic bronchitis to be 42% in smokers (Pelkonen et al. 2006). Increased prevalence rates of chronic bronchitis have been previously reported among former smokers, and the risk has been estimated to approach that of never smokers in five years (Brown et al. 1991, Troisi et al. 1995, Willemse et al. 2004). This is in line with our finding that former smokers abstinent for over ten years exhibited a lower risk for chronic bronchitis than those abstinent for less than two years.

Few studies have examined the association between light smoking and chronic bronchitis. A Finnish study found no increased risk associated with smoking <5 CPD (Pallasaho et al. 1999), whereas other studies have reported that smoking <10 CPD increases the risk of different respiratory symptoms (Amigo et al. 2006, Vianna et al. 2008). Substantial reductions in CPD have been described to decrease other respiratory symptoms (Hughes & Carpenter 2006, Pisinger & Godtfredsen 2007), not, however, chronic bronchitis (Simmons et al. 2005, Bohadana et al. 2006). This finding was confirmed here; the risk of chronic bronchitis among smoking reducers was at a similar level as that among daily moderate smokers.

This study supports the view that smoking reduction does not decrease the risk of chronic bronchitis, whereas permanently quitting smoking does. After quitting, the risk seems

to decline and is finally no longer significant relative to never smokers. Although no increased risk was observed for constant light smokers, a novel finding in this study was that light smoking measured at one time-point is associated with chronic bronchitis. The mean age of light smokers was 40.5 years in 1981, when chronic bronchitis was studied. Because chronic bronchitis morbidity correlates with pack-year exposure, the observed risk estimate for constant light smokers was possibly insignificant because their smoking history was rather short in 1981. With longer follow-ups, increased risks may be found also for constant light smokers.

6.2.4. COPD

This study demonstrated that moderate and heavy smokers, former smokers, those who reduced from moderate to light smoking, smokers relapsing to light / moderate smoking after quitting, and light smokers increasing their consumption to moderate smoking were at increased risk for COPD development. Light smoking, measured cross-sectionally in 1981, was also associated with COPD development, whereas constant light smoking over a six-year follow-up was associated with an increased risk for anticholinergic medication. The risk for COPD has not been estimated previously by taking into account changes that occur in smoking patterns longitudinally. The effects of light smoking and smoking reduction on respiratory health are only partially understood, and studies on their association with COPD are few. The novel findings of this study suggest that the risk of COPD is not altered by smoking reduction and that light smoking also increases the risk of COPD. Constant light smoking is likely to increase respiratory symptoms and pulmonary dysfunction since it was found to be associated with increased use of anticholinergic medication, although this use was not regular.

Successive measurements of smoking and the long follow-up of COPD development, based on national registry data of a large population sample, are factors increasing the reliability of these results. The incidence of COPD in this study was, however, much smaller than previously reported (Huhti & Ikkala 1980, Johannessen et al. 2005, de Marco et al. 2007). This is probably due to the disease definition used here: only symptomatic COPD patients are likely to end up using regular medication. Moreover, special reimbursement eligibility is given only to subjects suffering from severe disease.

Cross-sectional light smoking has been previously reported to be associated with mild COPD, defined by GOLD criteria, among subjects aged over 45 years (Lundbäck et al. 2003).

However, because the GOLD criteria may overdiagnose COPD in the elderly, spirometry-defined mild COPD may also reflect age-related decline in lung function. This study revealed that among the Finnish adult population, constant light smokers purchase combination preparations of ipratropium and $beta_2$ agonist significantly more often than never smokers. Medication use reflects increased pulmonary symptoms. Moreover, these subjects have sought a doctor's appointment since all medication purchases in Finland require a doctor's prescription. This the first time constant light smoking has been found to be associated with a COPD-related outcome. The results show that all levels of daily smoking may damage the lungs. Environmental factors were found in Study III to be important determinants of lung function, and all regular exposure to tobacco smoke appears to have detrimental effects on the lungs.

As many as 17% of the observed COPD cases were never smokers, although only 0.9% of all never smokers were affected. One possible explanation for this is that some of these 1975-1981 never smokers had taken up smoking during the follow-up. However, among those who attended again in 1990 (42%), all except one reported still being a never smoker. COPD in never smokers may be explained by other known risk factors, such as occupational exposure and passive smoking. Unfortunately, these factors were not assessed here and their relationship to COPD falls outside the scope of this thesis.

The mean age of participants was 40.2 years in 1981 and 67.2 years at the end of the follow-up in 2008. The 27-year follow-up of COPD development may have been too short to reveal all disease cases in the sample, as COPD incidence continues and cases accumulate beyond the age of 70 years (Pelkonen et al. 2006). Among constant light smokers and those who reduced from moderate to light smoking, no cases with a special reimbursement eligibility for COPD were observed. The average age of those who were light smokers in 1981 was only 61.5 years at the end of follow-up, thus, many of them may still develop COPD later in life. It is also possible that some study subjects suffered from mild, asymptomatic COPD during the follow-up and went undetected.

6.3. Methodological considerations

6.3.1. Representativeness of the study sample

This thesis aimed to assess different smoking patterns and their effects on pulmonary health at a population level. Participants of the Older Finnish Twin Cohort, a population-based study

with high response rates (Kaprio & Koskenvuo 2002), formed an excellent and representative sample for this purpose. The study sample of this thesis was composed mainly of those subjects who attended the follow-up in 1975 and again in 1981. In 1990, the invitation to participate was sent only to pairs born in 1930-1957 to avoid bias caused by increased morbidity in the older age group. A study based on the same cohort found that smoking and poor life satisfaction predicted nonparticipation in 1990 (Korhonen et al. 2007). If smokers dropped out more often than nonsmokers also from the 1975 and 1981 surveys, the prevalence of smoking may have been slightly underestimated in this study.

Heritability of lung function was investigated in the FITSA subsample, which, by contrast, is not as representative as the initial cohort. Walking and traveling ability were among the inclusion criteria, resulting in a small sample of participants who were probably healthier than women of the same age in the general population. Ever-smoking prevalence among the initial FITSA sample was 13.6%, which is low compared with women aged 65-74 years nationally (20.1% in 2001). The study subjects were also physically more active and their self-perceived health was better than same-aged women in the population (Sulander et al. 2001). At baseline, 62% of FITSA participants reported intermediate and 34% high physical activity, and at follow-up, these proportions were 60% and 30%. The proportion of participants suffering from chronic diseases, such as coronary heart disease, hypertension, and type 2 diabetes, was also lower than in the national sample (Sulander et al. 2001). The drop-out rate was moderate when taking into account the advanced age of participants; of the never smokers who attended spirometry, 74% participated in all measures again at follow-up. The baseline lung function among subjects who dropped out from the follow-up was, however, poorer than average, so nonparticipation at follow-up may have affected the results.

6.3.2. Measurement of smoking

Formation of smoking categories was based on self-reported smoking patterns, and no biochemical validation was used. About 75% of nicotine is metabolized to cotinine, which is regarded as the best biomarker for detecting exposure to tobacco smoke (Vasankari et al. 2011). The proportion of smokers in the twin cohort (32% in 1975, 28% in 1981) was, however, very similar to that in the Finnish general population in the 1970s and 1980s (Helakorpi et al. 1999), suggesting that participants reported their tobacco consumption honestly. Alternatively, if reporting bias existed, it was similar to that in the population-based health behavior surveys conducted in Finland.

Earlier validation studies in Finland have shown that smokers report their smoking status accurately; among self-reported never smokers, only 2.0-2.7% have had serum cotinine levels suggestive of smoking (Laatikainen, Vartiainen & Puska 1999, Vartiainen et al. 2002). However, a recent study using biochemical verification of smoking found that 7% of self-reported never smokers were actually smokers. In that study, the serum cotinine level was actually a better predictor of obstruction than self-reported smoking status (Vasankari et al. 2011). Biochemical verification of smoking would certainly have been advantageous also in this study. However, the twins represent the general population and results on cotinine values in Finnish samples are applicable to the twin cohort participants as well.

In this study, further smoking subgroups were formed to reflect the change in smoking patterns over a six-year period. Many participants had the same smoking status in both years, some quit during follow-up, and some increased their consumption, while the proportions of smoking decreasers and low-rate smokers were smaller (see Figure 3, Methods). The mean age of participants was 40.2 (SD 13.4) years in 1981. As smoking patterns acquired in middle age are usually stable (Culverhouse et al. 2005), smoking patterns in 1981 were likely to describe subjects' exposure over a longer time span. Accordingly, these smoking subgroups describe the cumulative exposure more precisely than cross-sectionally measured smoking. This is an important strength of this study, where the aim was to investigate long-term exposure and how it predicts several lung health outcomes.

The lifetime number of pack-years is the most significant risk factor for COPD (Burrows et al. 1977, Rennard & Vestbo 2006). Pack-year exposure based on a single report of cigarette consumption is, however, often unreliable. The daily number of cigarettes is a reliable measure, and when analyzed longitudinally, it reflects the changes that occur in tobacco exposure. In Studies II and IV, the models examining chronic bronchitis and COPD were adjusted for age as well as for pack-years, unless the correlation between pack-years and smoking categories was ≥ 0.6.

In the FITSA study, history of ever-smoking was based on self-reports. The proportion of never smokers was 86% among the initial study population, which is higher than in a national sample of elderly women (Sulander et al. 2001). The possibility that some subjects might have falsely classified themselves as never smokers cannot be excluded. This concerns especially those participants who smoked decades ago and only for a short period.

6.3.3. Measurement of outcomes

6.3.3.1. Lung function

Spirometry is one of the most accurate means of measuring lung function, and it was performed in the FITSA study according to international guidelines. Instructions and encouragement were given by professional technicians and the maneuvers were practiced until at least two were acceptable. The spirometer was calibrated daily with a three-liter pump and was accurate to within 1%. The spirometry values are likely reliable and equivalent to subjects' true pulmonary function.

The obtained spirometry results cannot, however, be generalized without qualification to elderly females. First, the reported results were based on measurements among never smokers only, and second, the study subjects were probably healthier than women of their age in the general population. However, the sample was derived from the relatively isolated Finnish population. An isolated population exhibits less genetic variability and shares a more uniform environment than outbred populations (Peltonen, Palotie & Lange 2000). This can be considered an advantage, increasing the representativeness of the study sample.

The mean FEV1 and FVC levels were higher than the mean expected FEV1 and FVC levels, which is in line with the small prevalence of pulmonary diseases and high physical activity among participants. However, the mean FEV1/FVC ratio was lower than the mean age- and height-correlated reference value, which supports the view that in elderly subjects FEV1/FVC<70% may be a normal phenomenon (Kerstjens et al. 1997, Medbo & Melbye 2007). However, as the Finnish reference values can be reliably applied only to subjects aged under 65 years (Sovijärvi et al. 2006), the estimation of expected lung function values among FITSA participants may be inaccurate.

Although the annual decline in FEV1/FVC speeds up in subjects aged over 70 years (Kerstjens et al. 1997), in the study sample the mean FEV1/FVC was greater at follow-up (77.4%) than at baseline (74.4%). However, as the values of FEV1 and FVC were both smaller at follow-up than at baseline, the spirometry measures are likely reliable and the observed increase in mean FEV1/FVC is a result of a higher decrease in mean FVC relative to FEV1. The mean lung function values were slightly greater among MZ twins than among DZ twins, except the FEV1/FVC ratio at baseline, which was higher among DZ twins. This may be related to the fact that, on average, MZ twins live longer than DZ twins (Zaretsky 2003).

Comparison of the genetic models to the saturated models, however, showed that the means and variances in spirometry values did not significantly differ by zygosity.

Twins are born smaller and at earlier gestational age than singletons, and in some populations, young adult twins have been reported to remain shorter and lighter than singletons (Pietiläinen et al. 1999, Silventoinen et al. 2008). As lung development lasts until young adulthood, smaller body size in twins may also result in smaller maximum lung capacity. Female MZ twins aged 45-65 years have also been reported to be slightly lighter than DZ twins and singletons (Andrew et al. 2001). The genetic models used in Study III were all adjusted for BMI or height, however, the average body size of the twin sample was not compared with that of elderly females in the general population.

Among strengths of the study design were exclusion of smoking effect and repeated spirometry measures. Controlling for the smoking-gene interaction was possible by including only never smokers in the study. Repeated lung function measurements allowed estimation of the changes in the proportions of genetic and environmental effects over time and examination of the correlations between baseline and follow-up estimates.

6.3.3.2. Chronic bronchitis

The diagnosis of chronic bronchitis is based on self-reported symptoms of long-standing cough and sputum production. The MRC question series is the appropriate way to define chronic bronchitis (British Medical Research Council 1960, Bobadilla et al. 2002) and was used also in the twin cohort questionnaires to identify subjects with chronic bronchitis.

The diagnosis of chronic bronchitis is based on self-reported symptoms, and no common objective way exists to measure the disorder. Accordingly, false-positive diagnoses may have occurred in, for example, cases of bronchiectasis, long-standing sinusitis, or untreated asthma (Bobadilla et al. 2002), where chronic cough and sputum production are sometimes present. In the twin cohort questionnaire, asthma was screened as a physician-made diagnosis, whereas bronchiectasis and sinusitis were not screened. The proportion of subjects with asthma among those suffering from chronic bronchitis was 6% in 1975 and 15% in 1981. The prevalence of bronchiectasis started to decline in Finland in the 1960s, and has been a rare condition since the 1980s (Säynäjäkangas et al. 1998).

A Swedish study interviewed and performed spirometry on subjects who had first completed questionnaires about respiratory symptoms. Of subjects reporting chronic productive cough, 17% actually had asthma, and elderly smokers were likely to underreport

their symptoms of chronic bronchitis on the questionnaire (Lundbäck et al. 1993). Thus, the high proportion of asthmatics in the chronic bronchitis group in 1981 may reflect increased susceptibility to cigarette smoke or false-positive diagnoses of chronic bronchitis among asthmatics. A recent Finnish study conducted among asymptomatic smokers revealed that 62% actually suffered from chronic cough or sputum production (Toljamo et al. 2010). As shown by these studies, smokers are not always aware of their respiratory symptoms or may deny them. Thus, underestimation of chronic bronchitis due to underreporting cannot be ruled out in Study II.

6.3.3.3. COPD

COPD was defined in Study IV as regular anticholinergic use (at least two purchases of anticholinergic inhalation preparations from the pharmacy at a maximum of 99-day intervals) or a diagnosis of COPD, chronic bronchitis, or emphysema entitling to special reimbursement eligibility. The anticholinergic medication criterion revealed 416 COPD cases, whereas 112 subjects were given a special reimbursement eligibility due to COPD, chronic bronchitis, or emphysema.

The main limitation of the COPD definition used in Study IV is that mild COPD and asymptomatic cases were likely excluded. First, the current care guidelines recommend medication use only for symptomatic COPD patients, and those with mild symptoms are sometimes treated with only $beta_2$ agonists. Second, anticholinergic medication, as most other medications in Finland, cannot be purchased from a pharmacy without a physician's prescription. As anticholinergic compounds are generally used to treat no conditions other than COPD, a medical examination and suspicion or diagnosis of COPD precede regular anticholinergic use. Accordingly, this is not likely to occur unless subjects become concerned about their pulmonary symptoms – and COPD patients easily become accustomed to their early symptoms (Toljamo et al. 2010). Third, special reimbursement eligibility for COPD medication is given only to subjects suffering from severe COPD as defined in lung function testing. Taking into account these limitations, the disease definition likely did miss some cases and falsely classified some affected subjects as healthy. The possibility of false-positive diagnoses, by contrast, is low.

Medications used during hospitalization periods or purchased abroad or in the pharmacy without presenting a health insurance card are not tracked in the SII. Accordingly, the disease definition used in Study IV may have missed such medication use. However, it is

unlikely that COPD patients would be admitted to hospital, treated there, but not continue anticholinergic medication afterwards. It is also uncommon not to present the health insurance card, which subsidizes 42% of the medication price. However, subjects with a special reimbursement eligibility due to another pulmonary disease, such as asthma or cystic fibrosis, who later developed COPD were more likely missed. The special reimbursement criterion may not have detected such persons because they do not need another certificate in order to receive a special reimbursement for pulmonary medications. In the twin cohort data, 19% of defined COPD cases had a special reimbursement eligibility for asthma. Indeed, asthma may evolve into COPD, and the coexistence of asthma and COPD, where patients have features of both, is common among elderly subjects (Gibson & Simpson 2009). One-fifth of elderly Finnish asthmatics use anticholinergics, suggesting that they also express features typical to COPD (Ikäheimo et al. 2005).

Among strengths of this follow-up of COPD development is the use of national registries, where drop-outs occur only if people die or move abroad. A spirometry follow-up would have resulted in a more accurate diagnosis; however, more drop-outs, especially among elderly subjects, would have occurred. Anticholinergic purchases registered by the SII have been used also previously to define COPD (Tynkkynen et al. 2009). Medication purchases are well covered in the SII because reimbursement for medication price is obtained when the health insurance card is presented at the pharmacy, and the purchase is then recorded. Regular anticholinergic medication, recommended by the national guidelines for COPD, is rarely used for other conditions. In Finland, physicians are educated in five medical schools with similar methods, and national treatment recommendations are carefully followed (Jousilahti et al. 2007).

6.3.4. Confounders

Most variables, e.g. alcohol use and physical activity, were screened with detailed, sometimes multiple questions, making the reported characteristics reliable (see Methods for detailed description). More unreliable variables include inhalation pattern, which was dichotomized as 'yes' or 'no'. However, only 6-7% of current smokers reported not inhaling in 1975 and 1981. Lifetime pipe or cigar smoking was defined as having smoked at least 3-5 packages of pipe tobacco or at least 50 cigars / 75 cigarillos. In 1975, 18% of the study population reported having ever smoked pipe or cigars. However, only 11.5% reported having smoked pipe or cigars regularly. These variables were used only in Study I when examining the characteristics

of light and heavy smokers. Because no data were available on daily amount of pipes and cigars smoked, the exposure could not be evaluated similarly as smoking in Studies II and IV. Smokeless tobacco use was not measured; however, its use in Finland during the 1970s and 1980s was rare (IARC Working Group on the Evaluation of Carcinogenic Risks to,Humans 2007).

Asthma and allergic rhinitis were screened by asking whether subjects had a diagnosis set by a physician. This may have resulted in underestimation of asthma and allergic rhinitis since not all affected subjects are diagnosed. However, accurate diagnosis of asthma cannot be performed in a questionnaire study; instead, this would require lung function measurements.

In Study III, participants' characteristics were measured in 2000 and again in 2003. Physical activity and disease status were self-reported. Physical activity and morbidity were assessed with multiple specific questions. Unfortunately, no post-bronchodilator spirometry was performed, which would have resulted in more accurate asthma diagnoses compared with self-reports. Possibly, elderly subjects may have overestimated their activity and capacity if they recently had experienced limitations in their performances. Difficulties in accepting one's reduced capacity may cause overestimation of self-reported physical activity and underreporting of morbidity.

Passive smoking and occupational exposure were not assessed in the twin cohort questionnaires or in the FITSA study. However, they may be important environmental factors affecting lung function in never smokers. In Western countries, smoking is the main factor causing chronic bronchitis and COPD (Mannino & Buist 2007). Occupational exposure has, however, been estimated to account for 15% of the burden of COPD in Western countries (Balmes et al. 2003, Salvi & Barnes 2009), and similar rates have been reported in Finland (Nurminen & Karjalainen 2001). In addition, passive smoking increases the susceptibility for chronic bronchitis and COPD (Radon et al. 2002, Mannino & Buist 2007, Yin et al. 2007). One limitation of this study is that passive smoking and occupational exposure were not taken into account. They might have explained part of the disease burden among never smokers. In genetic modeling, passive smoking and occupational exposure were included as unique environmental effects, but their proportional impact among other environmental factors is impossible to distinguish.

6.3.5. Statistical analyses

6.3.5.1. Random-effects models

In Study II, after reshaping the data into longitudinal panel data, increased risks of chronic bronchitis were found for former and light smokers with a random-effects regression model. The random-effects model made use of all information available on smoking patterns and analyzed data from all three measurement points simultaneously. As random-effects model assumes that repeated measurements are independent of each other and that heterogeneity between subjects is partially explained by unobservable effects (Diggle et al. 2002). Further, random-effects models can take into account data on subjects who later drop-out from the study (Hedeker & Gibbons 1997). The initial logistic regression models analyzing smoking status in 1975 and incidence of chronic bronchitis in 1981 found no significantly elevated risks for former and light smokers, probably because of the smaller number of observations compared with the random-effects model. However, in the pooled panel data, it could not be ascertained whether smoking patterns in a certain year preceded subsequent chronic bronchitis. Nor could the changes in smoking patterns be taken into account in this data. However, the other statistical analyses used in Study II established the causal relationship between smoking and COPD, and also evaluated the changes in smoking patterns.

6.3.5.2. Discordant twin pair analyses

Discordant twin pair analyses were conducted in Studies II and IV. In Study II, 112 pairs were discordant for chronic bronchitis, whereas 15 pairs (12 DZ, 3 MZ) were discordant also for smoking. The analysis conducted among all pairs could be repeated separately only for DZ twins, because among the three MZ pairs, there were no pairs in which the never-smoker co-twin was affected. Among DZ twins, the association between smoking and chronic bronchitis remained significant, and it is likely that with a larger sample, a similar association would be observed also in MZ twins. In Study IV, the sample of the discordant twin pair analyses was probably again too small. There, 325 pairs discordant for COPD were observed, and the association with smoking was analyzed among MZ (n=81) and DZ (n=213) pairs separately. The risk estimates associated with smoking were significant only among DZ pairs; those in MZ twins were high, albeit nonsignificant. According to these results, the possibility that some of the associations between smoking and COPD are explained by genetic factors cannot

be ruled out. It is more likely, however, that nonsignificance among MZ pairs was obtained because of the relatively small sample size.

6.3.5.3. Genetic modeling

Heritability of lung function was studied among never-smoking elderly females. The sample was rather small and, unfortunately, some drop-outs occurred at follow-up. The drop-out rate was similar in MZ and DZ twins. Despite the limitations, the sample was well suited for quantitative genetic modeling. At the time of baseline measurements, the zygosity of the twins was ascertained from blood sample DNA, and acceptable spirometry was obtained for 157 MZ and 182 DZ twins. Similar measurements were performed at three-year intervals, enabling the analysis of the data with a Cholesky decomposition model. A potential source of bias is the baseline requirement that both twins had to participate. It may have resulted in exclusion of pairs in which one sister was unhealthy or unable to travel, and consequently, the twin similarity may have been overestimated.

In genetic modeling, correct estimation of the genetic and environmental components affecting a phenotype requires fulfillment of certain assumptions. These include equal environments for MZ and DZ twins and the absence of GxE interaction, GE correlation, and assortative mating (Posthuma et al. 2003).

Equal shared and unique environments assumes that no differences exist between the means within MZ and DZ twin pairs and that an equal degree of environmental variation is present within them. Equality of environmental effects was tested by comparing genetic models to saturated ones; as the means and variances of lung function did not significantly differ by zygosity, this assumption appears tenable. Assortative mating, meaning that individuals choose spouses that resemble themselves, is known to occur for many traits, e.g. intelligence (Posthuma et al. 2003). Assortative mating tends to increase the similarity of DZ twins relative to MZ twins. If the parents of the twins had similar lung function, then the spirometry values of the twins would be more similar than expected due to higher genotypic resemblance. Although spouse correlations for current smoking are high (Boomsma et al. 1994), assortative mating regarding smoking is unlikely to have affected the study subjects; at the time they were born, smoking among women in Finland was rare (Helakorpi et al. 2004). Instead, assortative mating based on social homogamy (Evans, Gillespie & Martin 2002), e.g. among farmers, is possible, but does not result in genotypically more similar lung function in offspring.

As suggested by earlier studies (Silverman et al. 1998, Wilk et al. 2000, Zeller et al. 2010), an important source of GxE interaction is that between smoking and genes determining lung function. This was taken into account by excluding ever smokers from the study. However, other factors, such as occupational exposure, have also been shown to modify the genetic expression of lung function (Pacheco et al. 2010), and these were not taken into account in Study III. GE correlation occurs, for instance, when parents transmit both genes and environment affecting certain phenotype, or when subjects actively select environments correlating with their genotype. GE correlation may be active, passive, or reactive, and it is usually difficult to measure. GE correlation increases the phenotypic variance at the population level (Posthuma et al. 2003). For example, children with genetically determined poor lung function may actively choose not to practice sports, which may impair their achievement of maximal lung capacity.

In addition to dominant and additive genetic effects and the interaction between genes and the environment, there is also an interaction between genes, i.e. epistatic effects between different loci. In classical twin modeling, the epistatic effects are modeled as part of A and D effects. Loci that are closely linked and inherited together, are included in the A effects, whereas interactions between loci at distant sites are modeled as part of D effects.

6.4. Implications of this thesis

6.4.1. Health implications

Light smokers often do not consider themselves true smokers, and consequently, they may go unidentified by healthcare professionals (Schane, Ling & Glantz 2010). Light smokers are rarely advised to quit smoking (Owen et al. 1995, Okuyemi et al. 2001). However, because they are often planning to quit (Owen et al. 1995, Zhu et al. 2003, Hyland et al. 2005) and are probably less nicotine-dependent than smokers with heavier consumption (Shiffman et al. 1990), light smokers would be more likely to benefit from cessation interventions. Cessation pharmacotherapy has not been studied in light smokers, and current guidelines do not support use of medication if the amount smoked is <10 CPD (Winell et al. 2007, Schane, Ling & Glantz 2010). However, information, motivation, and support, all of which increase the likelihood of quitting, should also be offered to light smokers (Winell et al. 2007, Grief 2011).

In addition to light and occasional smokers, healthcare professionals should also pay attention to subjects who report having reduced their smoking. Possible false beliefs about the

diminished health risks should be corrected, and reducers should be advised to quit smoking. As studies have shown that smoking reduction predicts cessation (Hughes & Carpenter 2006, Broms, Korhonen & Kaprio 2008), quitting interventions might be particularly successful among such smokers. Smokers should not be advised to reduce smoking unless the final goal is complete abstinence from tobacco (Hughes & Carpenter 2006).

From the public health point of view, there is a need for better health education about the risks of low-rate smoking patterns. Possible reasons for the increase in the proportion of low-rate smokers may be false beliefs about a 'safe level of smoking', or intended 'harm reduction'. Low-rate smokers may be unaware of the health risks to which they are exposed. Information should be targeted to groups in which low-rate smoking patterns are especially common: adolescents, students, pregnant women, and certain ethnic minorities (Owen et al. 1995, Okuyemi et al. 2002, Hyland et al. 2005, Levy, Biener & Rigotti 2009, Pierce, White & Messer 2009). Intensive screening of smoking and health education among pregnant women, in particular, would be profitable, as women appear to be more prone to the harmful effects of smoking than men (Sorheim et al. 2010, Foreman et al. 2011), and smoking during pregnancy is a severe health risk for the fetus. As smoke-free policies have had a marked impact on tobacco consumption rates (Helakorpi et al. 2004, Helakorpi 2008), greater smoking restrictions could result in further benefits.

Maintenance of good lung function in the elderly is necessary to prevent excess COPD morbidity. The previous finding that a large proportion of lung function is determined by environmental effects was supported in this study. Environmental factors other than smoking were found to have a significant influence on lung function in women aged 63-76 years. Disease processes, accidents, sarcopenia (Sharma & Goodwin 2006), occupational or second-hand smoke exposure (Kerstjens et al. 1997), low physical activity (Amara et al. 2001), and malnutrition (McKeever et al. 2008) are among the factors that may predispose to a decline in lung function and a concomitant worsening of overall functional capacity and health. Even in healthy elderly persons, multiple physiological modifications occur in the lungs over time (Chan & Welsh 1998), contributing to the enhanced decline of lung function after the age of 65 years (Kerstjens et al. 1997). One important factor worsening age-related decline is immobilization, which rapidly leads to disability and muscle waste in the elderly. Further, it is uncommon for elderly persons to regain the previous functional level (Suetta et al. 2007). Thus, physical activity, muscle training, and early and active rehabilitation when needed are important. Also, a good nutritional status helps to maintain lung function (McKeever et al.

2008). Furthermore, the observed association between mental and respiratory health (Goodwin et al. 2007) suggests that avoiding and treating mental problems may prevent excessive lung function decline.

6.4.2. Implications for future research

This study found that the prevalence of light smoking remained constant at 2.5% among the Finnish population between 1975 and 1990. In many other countries, a trend of increasing low-rate smoking patterns has been observed. In recent years, the daily number of cigarettes among regular smokers has decreased also in Finland; however, the proportion of occasional smokers has remained the same for decades. Further research in the current Finnish adult population would reveal whether the proportion of light smokers has changed since 1990.

The risk of COPD increases with pack-years of smoking, which is one reason why the disease particularly affects elderly subjects. Constant light smokers are likely to have, on average, a slower lung function decline than heavy smokers, and they may therefore develop chronic bronchitis and COPD later. In this study, the mean age of light smokers (identified from the 1975 study) was 34.5 years in 1981, when the incidence of chronic bronchitis was investigated, and 61.5 years at the end of the COPD follow-up. To fully understand the health risks associated with light and occasional smoking, lifelong follow-ups of such smokers are needed.

When assessing lung function heritability at two time-points, the genetic factors affecting lung function were rather constant, whereas new environmental factors emerged at follow-up. Further research is required to recognize such time-specific environmental risk factors, as well as to better understand the underlying mechanisms determining lung function. As lung function is likely influenced by complex relationships between genetic and environmental factors, gene-environment interaction studies are warranted to reveal these pathways.

7. Conclusions

This study assessed longitudinally the consistency of light smoking in the Finnish population and evaluated which characteristics describe this subgroup of smokers. The risks of chronic bronchitis and COPD were estimated according to changes in smoking patterns between 1975 and 1981, with a particular focus on light smoking and reduced smoking. Further, the proportions of genetic and environmental factors affecting lung function were estimated in elderly never-smoking Finnish females.

This study reveals that light smokers form a small, but stable subgroup among Finnish adult smokers. However, the finding that most light smokers quit or increase their smoking during follow-up indicates considerable turnover on an individual basis. Light smokers are more often female, young, well-educated, single, and exhibit a healthier lifestyle than heavy smokers. Targeting information about the health risks of low-rate smoking to groups with the above-mentioned characteristics seems reasonable. Efforts are required to motivate light smokers to quit. As light smoking is increasing globally, and smokers also in Finland consume fewer cigarettes than earlier, research on present-day Finnish light smokers is warranted.

The heritability of lung function was found to be lower among elderly never-smoking Finnish females than previously reported in other populations. The proportions of genetic and environmental factors affecting lung function were assessed longitudinally for the first time. Genetic factors influencing FEV1 and FEV1/FVC remained the same, whereas new genes emerged to explain part of the observed variance in FVC at follow-up. Environmental factors others than smoking were found to be important determinants of lung function in elderly females, suggesting that abstinence from tobacco is not enough to maintain good respiratory health. Drawing attention to possible external risk factors may help to maintain normal lung function in old age.

This study supports the view that regarding pulmonary disorders no safe level of daily smoking exists. The results provide strong evidence that reducing smoking to fewer than five cigarettes a day does not protect against chronic bronchitis or COPD. The disease risk actually seems to remain unchanged regardless of smoking reduction since moderate smokers and increasers exhibited similar risks for regular anticholinergic use. Thus, smoking reduction should not be considered an alternative to cessation. Further, this study demonstrated that also daily light smoking increases the risk of chronic bronchitis and COPD. The incidence of

chronic bronchitis was screened at an average age of 49 years, and the follow-up for COPD ended at an average age of 67 years. Although the prevalence of COPD is highest among the oldest age groups, the results show that smoking-induced respiratory disorders often occur already in middle-aged individuals. As COPD and chronic bronchitis are likely to cause significant disability and morbidity later in life, the possibility of their presence should be considered also in light smokers, regardless of age.

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	Change in smoking 1975-1981	Smoking categories	N (%)
Classification in Study II	Never / occasional → never	**Constant never smokers**	10,083 (46.80)
	Never / occasional / former→ occasional	**Occasional / non-daily smokers**	567 (2.63)
	Former → former	**Constant former smokers**	2,594 (12.04)
	Never → light / moderate / heavy	**Initiators**	354 (1.64)
	Never / occasional / light / moderate / heavy → Former	**Quitters**	1,960 (9.10)
	Former → light / moderate / heavy	**Recurrent smokers**	578 (2.68)
	Light → light	**Constant light**	104 (0.48)
	Moderate → moderate	**Constant moderate**	2,510 (11.65)
	Heavy → heavy	**Constant heavy**	1,048 (4.86)
	Increase in daily light / moderate smoking	**Increasers**	814 (3.78)
	Decrease in daily moderate / heavy smoking	**Decreasers**	548 (2.54)
	Misclassifications and illogical reports	**Others**	386 (1.79)
Classification in Study IV	Never → never	**Never smokers**	9,852 (45.7)
	Never / occasional → occasional	**Occasional smokers**	411 (1.91)
	Never → light / moderate, occasional → light / moderate / heavy	**Initiators**	341 (1.58)
	Former / light / moderate / heavy → former	**Former smokers**	4,130 (19.17)
	Moderate / heavy → moderate	**Moderate smokers**	2,906 (13.49)
	Never / former / light / moderate → heavy	**Increasers**	833 (3.87)
	Moderate → light	**Moderate → light**	176 (0.82)
	Heavy → light	**Heavy → light**	9 (0.04)
	Heavy → heavy	**Constant heavy smokers**	1,053 (4.89)
	Former → light / moderate, light → moderate	**Small increase**	617 (2.86)
	Light → light	**Constant light smokers**	109 (0.51)
	Misclassifications and illogical reports	**Others**	1,109 (5.15)
Total			21,546 (100%)

Appendix 1. Formation of smoking categories describing change in smoking between 1965 and 1981 in Studies II and IV. The final categories are presented in the middle column, initial smoking groups on the left, and group size on the right.