Twins studies as a model for studies on the interaction between smoking and genetic factors in the development of chronic bronchitis

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Abstract

Smoking is the main risk factor for COPD (chronic obstructive pulmonary disease) but genetic factors are of importance, since only a subset of smokers develops the disease. Sex differences have been suggested both in disease prevalence and response to environmental exposures. Furthermore, it has been shown that acquisition of ‘addiction’ to smoking is partly genetically mediated. Disease cases and smoking habits were identified in 44919 twins aged >40 years from the Swedish Twin Registry. Disease was defined as self-reported chronic bronchitis or emphysema, or recurrent cough with phlegm. The results showed that chronic bronchitis seems to be more prevalent among females, and that the heritability estimate for chronic bronchitis was a moderate 40% and only 14% of the genetic influences were shared by smoking.

Introduction

COPD (chronic obstructive pulmonary disease) represents an important and increasing burden throughout the world. The prevalence of chronic bronchitis and COPD increases with age. Cigarette smoking is a major risk factor for COPD, but not all smokers develop COPD [1], and, furthermore, COPD occurs also in never-smokers [2]. Smokers who develop COPD seem to be genetically more susceptible to the deleterious effects of cigarette smoke than smokers who do not develop COPD [3,4]. COPD has been shown to cluster in families [5,6]. A recent study indicates familial aggregation of airway wall thickening and emphysema [7]. The best described genetic process involved in COPD is α₁-antitrypsin deficiency, where the impairment of the ability to produce a protective protein is associated with risk for premature development of emphysema, even in non-smokers [8,9]. It does, however, account for only 1–2% of COPD cases [10]. It is also known that dependence on tobacco is a complex behaviour, with both genetic and environmental factors contributing to population variation [11]. Women seem to be more predisposed to suffer from the adverse respiratory consequences of tobacco smoking [12]. Overall the specific mechanisms are not fully understood and it is likely that many genes and environmental factors interact in the development of COPD [13].

COPD and inflammation

Results from the Lung Health Study [14] showed that, on average, for every 10% decrease in FEV₁ (forced expiratory volume in 1 s), all-cause mortality increased by 14%, cardiovascular mortality by 28% and non-fatal coronary events by almost 20%, after adjustment for confounders such as age, sex, smoking status and treatment assignment. Systemic inflammation has been put forward as a possible explanation for the high prevalence of co-morbidities such as CVD (cardiovascular disease) in patients with COPD. Studies have shown that the inflammatory markers plasma fibrinogen and CRP (C-reactive protein) are associated with CVD and all-cause morbidity as well as an increase in COPD mortality [15–17]. Increase in inflammatory plasma markers have been observed in patients with COPD, as well as in healthy subjects with reduced lung function [18–25]. Some previous studies on CRP and lung function have given mixed results [26–28]. A recent 25-year follow-up on 5247 apparently healthy men found the incidence of COPD hospitalizations to be associated with the number of elevated inflammation-sensitive plasma proteins (fibrinogen, ceruloplasmin, α₁-antitrypsin, haptoglobin and orosomucoid) [29]. The

Key words: chronic obstructive pulmonary disease (COPD), heritability, inflammatory marker, multi-slice computed tomography (MSCT), smoking, twin

Abbreviations used: COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; CVD, cardiovascular disease; DLCO, diffusion capacity of the lung for carbon monoxide; D2, drygyle; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; MSCT, multi-slice computed tomography; MZ, monozygotic; SALT, Screening Across the Lifespan Twin; STR, Swedish Twin Registry.

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role of these inflammatory markers in clinical practice is still uncertain. Thus it would be of great interest to examine inflammatory markers in different phenotypes of COPD.

**Twin methods**

Twin studies can be used to estimate genetic influence. Analyses of the relative importance of genes and environments for a phenotype can be carried out with traditional quantitative genetic methods that are well developed for twin studies [30]. The classical twin method is based on the fact that MZ (monozygotic) twins share 100% of their genome, whereas DZ (dizygotic) twins share on average 50% of their segregating genes. Historically, concordances were computed separately for MZ and DZ pairs. When MZ concordances are greater than DZ concordances, genetic influences are indicated. Tables can be used to estimate heritability from concordances; however, these estimates are hampered by a number of limitations [31]. Information about shared genetic and environmental influences allows one to use a set of linear structural equations and fit models over all types of twins to best describe the causes of variation in a phenotype. The total variance in the trait can be partitioned into genetic variance (A), common environmental variance, including shared (familial) environmental variance (C) and unique environmental variance (E). In order to estimate the parameters of interest, the equation for one of the twins can be written as:

\[ V_p_1 = a^2A_1 + c^2C_1 + e^2E_1 \]

where \( V_p_1, A_1, C_1 \) and \( E_1 \) are the total phenotypic variance, additive genes, shared environments and unique environments respectively for the first twin in the pair. A similar equation can be written for the second twin. The theoretical expectations for variance and covariance (Cov) within twin pairs can be described with the following equations:

\[ V_p = a^2 + c^2 + e^2, \]

\[ \text{Cov MZ} = a^2 + c^2, \]

\[ \text{Cov DZ} = 0.5a^2 + c^2. \]

The parameters \( a, c \) and \( e \) can then be estimated with maximum likelihood methods.

Similar to quantitative genetic methods, the co-twin control method takes advantage of the fact that MZ and DZ twins share different degrees of genetic relatedness.

**Examples based on the STR (Swedish Twin Registry)**

The STR contains information on more than 80,000 twin pairs born from 1886 to 2000 [32]. Between 1998 and 2002, all living twins in the STR born in 1958 or earlier were contacted using a computer-assisted telephone interview in the SALT (Screening Across the Lifespan Twin) study [33]. The SALT interview included introductory items concerning zygosities and a checklist of common diseases. The registry, which today has developed into a unique resource, was first established in the late 1950s to study the importance of smoking and alcohol consumption on cancer and CVDs while controlling for genetic propensity to disease. Since that time, the Registry has been expanded and updated on several occasions, and the focus has similarly broadened to include most common complex diseases. Today the STR includes practically all twins born in Sweden since 1886.

Twin registry has been the basis of a number of co-twin control studies with an experimental design to prove that different functional aspects of the respiratory system are influenced by genetic factors. For example, one very basic protective mechanism for the lung, namely tracheobronchial clearance, is highly similar in MZ twins. Smokers in MZ smoking-discordant twin pairs had worse tracheobronchial clearance compared with their twin partner. These results have important consequences, because tracheobronchial clearance is closely related to development of symptoms of chronic bronchitis [34,35].

**Airways and twins**

Heritable effects have been found to explain 26% of the variation in susceptibility to lung cancer [36]. The Young Twin study of 7–9-year-old twins has evaluated genetic-, environmental- and gender-specific factors of importance for atopic disease [37]. We found interesting gender differences with boys presenting more symptoms from airways (asthma and allergic rhinitis) and girls more symptoms from the skin including eczema. Genetic effects accounted for approx. 70% of the variation in liability for asthma and somewhat less for the other atopic diseases (hay fever, eczema and urticaria).

Twin studies have suggested that genetic factors are of importance in individual differences in lung function [38–40]. Cross-sectional data on pulmonary function indicate that environmental factors may become gradually more important with age. Pulmonary function measured in the SATSA (Swedish Adoption/Twin Study of Ageing), making use of twins reared apart, clearly showed that genetic factors are important for pulmonary function in elderly individuals (50 years and older) [40]. This is especially interesting as pulmonary measures are associated with clinical disease and mortality. Furthermore, longitudinal data indicate that pulmonary function predicts cognitive function 6 years later, and genetic factors are important for this association [41]. However, as normal and pathological lung function decline is not necessarily promoted by the same factors, these results cannot be directly translated to a population with airway disease.

**Chronic bronchitis in the STR**

A series of questions related to respiratory symptoms and chronic bronchitis was also included in the SALT study. Special emphasis was put on diagnostic items that could determine whether a twin was likely to have a disease, rather than simply asking the twin if he or she had a disease. Disease cases and smoking habits were identified in 44,919 twins aged >40 years. The crude prevalence of chronic bronchitis was 7.1%. Prevalence estimates, stratified on smoking habits,
Heritability estimate for chronic bronchitis was 40% and only 14% of that were shared by smoking. The heritability estimate for chronic bronchitis was 40% and only 14% of its genetic influences were shared by those for smoking (Figure 1). From the results of the present study, we concluded that heritability has a moderate influence on the development of chronic bronchitis, and that the genes that are involved are largely independent of those related to smoking habits. We have also observed that chronic bronchitis seems to be more prevalent among females than males [42]. Chronic bronchitis is closely associated with COPD. However, to further study COPD, determination of lung function is necessary.

Ongoing studies

Spirometry and the single breath test of DLCO (diffusion capacity of the lung for carbon monoxide)

A sample of 392 twins was invited for lung function testing with spirometry. Disease concordant and discordant twins were prioritized over symptom-free twin pairs. Technically acceptable FEV1 and FVC (forced vital capacity) measurements were performed by 378 individuals in 181 complete pairs. The corresponding figures for acceptable DLCO measurements were 375 individuals in 178 complete twin pairs.

Zygosity

Zygosity of the sex-like pairs was determined by the use of a set of DNA markers from blood drawn at the clinical testing. Blood samples were not available for both members in 14 pairs, and zygosity information for these twins was instead obtained at the time of registry compilation on the basis of questions about childhood resemblance. Four separate validation studies using serology and/or genotyping have shown that with these questions 95–98% of twin pairs are classified correctly [33].

Systemic biomarkers

Plasma and serum biomarkers will be analysed in both COPD and healthy subjects to investigate different aspects of systemic inflammation. The biomarker levels will be compared in COPD versus healthy individuals, and correlated to different clinical data and MSCT (multi-slice computed tomography) parameters.

MSCT

Thin slice computed tomography was offered to twin pairs where at least one had an FEV1/FVC below 90% of predicted. In total, 108 MSCT images were analysed using software for detection and visualization of emphysema (YACTA) and the emphysema index [% relative area at −950 HU (Hounsfield units)]. Within-pair differences will be compared for smoking concordant (n = 14 pairs, both ever-smokers) and smoking discordant (n = 5 pairs, one never-smoker and one ever-smoker) pairs. Additional computed tomography scans have been performed in the affected twins and their respective twin partner to increase numbers but they have not been analysed yet.

Concluding remarks

Twin studies have been proved to be useful for quantitatively estimating the genetic influence of disease. For respiratory disease, results have been published showing not only clear heritability regarding different aspects of allergic disease, but also interesting gender differences. Familial aggregation has been shown for COPD, emphysema and airway wall thickening. The specific mechanisms explaining heritability are not fully understood. α1-Antitrypsin and tracheobronchial clearance are related to the development of chronic bronchitis. There is also clear heritability for pulmonary function in population-based samples. Tobacco smoking is the main risk factor for COPD. It is furthermore known that dependence on tobacco is a complex behaviour, with both genetic and environmental factors contributing to population variation. We have in the present study shown a moderate heritability for chronic bronchitis in a study based on the STR. Only 14% of the genetic influences were shared by those for smoking. There are further interesting data, which will be analysed to evaluate gender differences regarding the effect on pulmonary function and susceptibility to develop COPD.

Several studies give fairly strong support to the theory that inflammatory biomarkers among healthy subjects have a prognostic value [15–17]. There are also results indicating differences between healthy subjects and persons with late stage COPD [18–25]. At the ERS (European Respiratory Society) Congress in Berlin 2008, Wollmer et al. [43] reported their finding that urine-desmosine, a marker of lung tissue destruction, was higher (P = 0.009) and DLCO was lower (P < 0.001) in COPD patients, n = 33, compared with lung-healthy subjects, n = 24. This indicates the possible use of urinary samples. It has been suggested that COPD and all
its co-morbidities should come under the 'chronic systemic inflammatory syndrome' [44]. We believe that further studies on twins might add useful knowledge and that there is a need for better description of phenotypes.

Our ongoing study is based on the general population and not on hospital-based patients and includes less severe disease compared with their twin partners. We will evaluate heritability for chronic bronchitis, sex differences in the relative impact of genetic factors on lung function and the role of genetics in the development of emphysema.

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**References**


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