Respiratory twin studies

Ph.D. Thesis

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1. Introduction

Twin studies have been a valuable source of information about the genetic basis of complex traits. Comparing monozygotic (MZ) and dizygotic (DZ) twins by classical twin study, genetic background of diseases can be evaluated in susceptibility to a disease. In case of a heritable disease, MZ twin pairs are more concordant than DZ twins. Additive genetic influence (A), common familial environmental factors (C) and unique environmental factors (E) can be estimated by univariate quantitative genetic ACE models.

Twin studies in Hungary date back to 1970s on the basis of three different databases, all of them through the efforts of Endre Czeizel and Júlia Métneki. The Budapest Twin Registry (BTR) was launched in 1970, the BTR offered a unique opportunity for scientific research. In the early 1980s, a second volunteer adult twin registry was initiated. The third database, the Hungarian Congenital Abnormality Registry (HCAR), established in 1970, included personal and medical data of multiple births. Unfortunately, institutional and administrational changes led to the complete discontinuation of the registries in the 1990s. Therefore, there was an increasing need to establish a new twin regisry in order to start the respiratory twin studies.

The respiratory system is a biological system that introduces respiratory gases to the interior and performs gas exchange. Pulmonary function tests provide valuable information about the lung's integrated mechanical function, chest wall and respiratory muscles. The most common variables are forced vital capacity (FVC) and forced expiratory volume at 1 second (FEV_1). Heritability of lung function has been investigated in twin and family studies with various results. In most studies, the heritability of FEV_1 ranged between 10% and 77% and one of FVC ranged between 26% and 91%.

The relationship between impaired lung function and atherosclerosis, cardiovascular morbidity and mortality has been poorly investigated. Reduced pulmonary function is associated with increased incidences of cardiovascular disease and death. However, no data are available on the relation of impaired lung function and novel cardiovascular phenotypes, such as wave reflection or arterial stiffness. Arterial stiffness, characterized by aortic pulse wave velocity (PWV) and some limited extent: augmentation index (AIx), measure of wave reflection, have independent predictive values for cardiovascular events.

Apart from lung function, secondhand smoke (SHS) is also a phenotype of interest in respiratory studies. SHS is a complex mixture of the gases and particles given off by the burning end of a cigarette, pipe or cigar. These particles are in the fine to ultrafine particle size range (0.02 μm–2 μm) and have been shown to be inhaled deep into the lungs and to cause an array of adverse health effects including cancer, heart attacks and asthma. Epidemiological studies found association between cigarette smoking and psychiatric disorders in context with adolescents' regular smoking, such as conduct disorders, attention-deficit/hyperactivity disorder, internalizing disorders (depression, anxiety) and aggression. Several twin studies investigated the possible role of genetic factors on nicotine dependence and withdrawal. However, little data are available on SHS of MZ and DZ twins.

2. Aims

Since twin studies reveal the proportion of genetic and environmental contribution of a trait, and how the two interact, this model can be applied in a respiratory setting as well. Furthermore, studying twins helps to draw conclusions concerning psychosocial aspects.

Our aims can be summarized in the following points:

1. To establish the Hungarian twin registry and describe the characteristics of the voluntary twin sample whose individuals will be involved in respiratory twin studies.

2. To assess the heritability of lung function, phenotypic correlations between pulmonary function (FEV1, FVC) and hemodynamic variables. Furthermore, to determine whether there is a shared genetic relation between lung function and arterial stiffness and wave reflection. We hypothesized that there is a common genetic background between lung function and arterial stiffness or wave reflection.

3. Third, we were specially interested how secondhand smoke exposure effect monozygotic and dizygotic twins in various indoor public places. Even if the heritability of smoking characteristics is well described, to date, there is no information regarding smoking and secondhand smoke characteristics of twins and its psychosocial aspect. Therefore, the last purpose of the investigation was to assess the smoking habits and sensibility to SHS exposure of twins comprehensively in a relatively large twin cohort.

3. Subjects and methods

3.1. Subjects of the twin studies

In 2006, we began an effort to revive the Hungarian Twin Registry with Levente Littvay and Júlia Métneki. The old volunteer registry and the twin gatherings were at the foundation of the new volunteer twin registry. Additionally, we are augmenting this list with social media presence, a continuous push in the more traditional media, and via a website (http://www.ikrek.com). In the respiratory twin studies, 151 monozygotic and 62 dizygotic healthy adult twin pairs were involved in Hungary and in the United States.

3.2. Study design

Adult MZ and DZ twins were recruited. Zygosity was assigned according to a seven-part self-reported response (>99% accuracy). Hungarian subjects were enrolled from the Hungarian Twin Registry and investigated at Hungarian twin festivals (Ágfalva and Szigethalom) and in two large hospitals in Budapest (Semmelweis University Department of Radiology and Oncotherapy; Military Hospital Department of Cardiology) in 2009 and 2010. American twins were tested at the Twins Day Festival in Twinsburg, OH, USA. Subjects completed a questionnaire separately of each other on the spot concerning smoking and SHS exposure characteristics.

3.3. Pulmonary function assessment

Lung function was assessed by dynamic spirometry (Minispir Waukesha, WI, USA). FVC and FEV_1 was expressed in absolute (measured) values and as percentage of predicted (based on the subject's age, height, sex, country), using the reference values.

3.4. Hemodynamic measurement

Aortic pulse wave velocity (PWV) - a measure of arterial stiffness - and brachial and aortic augmentation indices (AIx) - measures of arterial wave reflection - were assessed by a clinically validated oscillometric device (TensioMed Arteriograph, TensioMed Ltd., Hungary). This method enables the calculation of these parameters from oscillometrically recorded pressure waves on the brachial artery (pulse wave analysis).

3.5. Statistical analysis

3.5.1. Data analysis

Descriptive analysis (mean ± standard deviation for continuous variables, percentage for categorical variables) for the traits of interest was conducted using SPSS (SPSS 17.0 for Windows; SPSS, Chicago, IL). Differences between genders, zygosity and countries were calculated using independent-sample t-tests.

3.5.2. Estimating genetic influences on lung function and PWV, AIx

All analyses were corrected for age, gender, country and smoking. Univariate quantitative genetic modeling was performed to decompose the phenotypic variance of the considered parameters into heritability (A), shared (C), and unshared (E) environmental effects (ACE analysis).

3.5.3. Estimating the correlation between lung function and PWV, AIx

Correlation coefficients between pulmonary function (FEV_1, FVC), aortic PWV and AIx were calculated to measure the strength and the direction of their relationship.

3.5.4. Genetic covariance between lung function and PWV, AIx

A bivariate Cholesky decomposition was used to derive the magnitude of covariation between the investigated respiratory function and hemondynamic phenotypes (PWV, AIx) and to estimate what proportion of this correlation is attributable to common underlying genetic and environmental factors. In order to estimate the amount of overlap between

genes or environment that influences the two parameters, genetic and environmental correlations between those phenotypes were calculated.

4. Results

4.1. Characteristics of the Hungarian twin registry

Currently the Hungarian Twin Registry consists of 310 twin pairs (or multiplets - 65% MZ, 15% DZ, 20% opposite-sex DZ - 6 triplets, 1 quadruplet, 70% female, mean age 44±16 years). In the current database, we have data on risk factors, diseases and surgeries, various anthropometric and cardiovascular phenotypes in addition to the contact information (including address, telephone and email). Volunteers of the Hungarian Twin Registry were encouraged to participate in the respiratory measurements of the International twin study 2009.

4.2. Clinical characteristics and measures

In the analysis of the relation of lung function and hemodynamic variables, 196 healthy Hungarian and American twin pairs (154 monozygotic and 42 dizygotic; age 43±17 years±standard deviation) were included. In the analysis of smoking and SHS exposure characteristics of twins, 161 Hungarian and 50 American twin pairs (151 monozygotic and 62 dizygotic including 40 opposite-sex DZ pairs; mean age 43.8±16.5 years±standard deviation) were included.

4.3. Results of the lung function twin study

4.3.1. Heritability analysis of pulmonary function and PWV, AIx

Genetic factors appear to contribute, at least in part, to the pulmonary function, arterial stiffness and wave reflection parameters, as the MZ twins had higher intrapair correlation compared to that of DZ twins (Table 1).

Table 1. Age, gender, country and smoking-year adjusted genetic and environmental variance component parameter estimates and 95% confidence intervals of the Best-Fitting Univariate ACE models

Measure	**A**	**C**	**E**
FVC, % predicted	0.45 (0.00-0.66)	0.14 (0.00-0.56)	0.41 (0.31-0.52)
FEV_1, % predicted	0.28 (0.00-0.67)	0.31 (0.00-0.59)	0.41 (0.27-0.55)
Measured FVC	0.68 (0.20-0.81)	0.08 (0.00-0.55)	0.24 (0.17-0.32)
Measured FEV_1	0.73 (0.45-0.85)	0.00 (0.00-0.55)	0.26 (0.17-0.37)
Aortic AIx	0.58 (0.10-0.75)	0.10 (0.00-0.57)	0.32 (0.24-0.44)
Brachial AIx	0.55 (0.08-0.74)	0.13 (0.00-0.57)	0.33 (0.24-0.45)
Aortic PWV	0.50 (0.25-0.68)	0.00 (0.00-0.00)	0.50 (0.33-0.73)

A indicates heritability; C, shared environmental variance component; E, unique environmental variance component; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; AIx, augmentation index; PWV, pulse wave velocity.

4.3.2. Phenotypic correlation between pulmonary function and PWV, AIx

Phenotypic correlation ranged between -0.12 and -0.17 ($p<0.05$) between measured pulmonary function values and both brachial and aortic augmentation indices, suggesting that better measured lung function corresponds to lower AIx values (Table 2). In contrast, FVC and FEV_1 values showed no significant phenotypic correlations with aortic PWV. A possible genetic covariance of FEV_1, FVC and AIx was estimated by bivariate Cholesky decomposition model. Since measured and percent predicted FVC and FEV_1 values showed no significant phenotypic correlation with aortic PWV, the influence of common genetic and environmental factors on those relationship was not investigated.

Table 2. Bivariate family, age, sex, population corrected phenotypic correlations and 95% confidence intervals with or without smoking adjustment from a bivariate structural equation saturated model of a genetic covariance decomposition model between lung function, wave reflection and arterial stiffness

		Brachial AIx	Central AIx	Aortic PWV
Non smoking adjusted	FVC, % predicted	0.038 (-0.079, 0.156)	0.037 (-0.081, 0.155)	-0.030 (-0.145, 0.085)
	FEV_1, % predicted	0.011 (-0.107, 0.129)	0.009 (-0.109, 0.128)	-0.055 (-0.172, 0.061)
	Measured FVC	-0.147* (-0.267, -0.027)	-0.150* (-0.271, -0.030)	0.000 (-0.120, 0.119)
	Measured FEV_1	-0.162* (-0.281, -0.043)	-0.166* (-0.285, -0.046)	-0.007 (-0.128, 0.115)
Smoking adjusted	FVC, % predicted	0.063 (-0.055, 0.181)	0.062 (-0.056, 0.180)	-0.017 (-0.134, 0.099)
	FEV_1, % predicted	0.040 (-0.078, 0.159)	0.039 (-0.080, 0.157)	-0.041 (-0.160, 0.077)
	Measured FVC	-0.121 (-0.243, 0.001)	-0.123 (-0.246, -0.001)	0.012 (-0.108, 0.133)
	Measured FEV_1	-0.133* (-0.254, -0.012)	-0.126* (-0.246, -0.006)	0.009 (-0.114, 0.131)

AIx, augmentation index; PWV, pulse wave velocity; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second

* $p<0.05$

4.3.3. Genetic covariance of FEV_1, FVC and augmentation indices

Additive genetic components showed no significant influence for the covariance between lung function values and augmentation indices.

4.4. Smoking and secondhand smoke characteristics of twins

4.4.1. Comparison of smoking habits, smoking characteristics, secondhand smoke exposure, and local home, car and workplace smoking regulations of monozygotic and dizygotic twins

MZ twins reported higher rate of everyday and regular smoking during for the duration of at least one year ($p<0.05$). MZ twins started smoking 1.8 years earlier compared to dizygotic twins (17.7±4.1 versus 19.5±5.1 years), however, the difference was not statistically significant ($p=0.08$). Dizygotic twins smoked non-significantly more cigarettes for a significantly longer duration ($p<0.01$). Dizygotic twins suffered from higher amount of regular parental smoking exposure in childhood in their flats ($p<0.05$) compared to MZ twins. No difference was observed in the disturbing effect of secondhand smoke and in the daily secondhand smoke exposure at home, workplace or other areas independently of individual smoking status between MZ and DZ twins. Interestingly, significant difference was detected in smoking regulations both at home and workplaces between MZ and DZ twins ($p=<0.005$). More restricted smoking zones (rooms) were reported by MZ twins in the venues visited by them. The presence of building smoking regulation and SHS exposure in living space and cars did not differ across zygosity.

4.4.2. Secondhand smoke exposure in local bars and pubs, restaurants, cafés and public transportation venues of monozygotic and dizygotic twin pairs

No significant differences were reported in the prevalence of smoking regulations in local bars and pubs, restaurants and cafés and public transportation venues regarding zygosity. The frequency of visits at these venues was not different across zygosity except local transportation venues ($p<0.05$). Monozygotic twins spent significantly more time occasionally in bars and pubs than DZ twins ($p<0.05$) which was not present in additional investigated venues. Monozygotic twins reported significantly less smoke pollution in both local bars/pubs and restaurants/cafés ($p<0.01$). This difference was not present regarding the public transportation venues. Finally, no significant difference was observed in smoking prevalence at these venues across zygosity.

5. Conclusions

5.1. The first Hungarian Twin Registry was established in Budapest in 1970 through the mandatory reporting of multiple-births. In the 1980s a second, volunteer adult registry was also founded. Unfortunately, both registries ceased to exist in the 1990s. Efforts started in 2006 to revive a Hungarian twin registry. Currently, the voluntary Hungarian Twin Registry consists of 310 twin pairs and multiplets. Current research focuses (among others) on cardiovascular and respiratory health and yielded multiple awards and publications. Efforts are on the way to expand into social, psychological, obesity and further respiratory studies. Multiple researches were carried out, such as respiratory ones.

5.2. Lung function is strongly heritable. Measured FVC and FEV_1 is phenotypically, but not genetically, associated with augmentation index. No association between lung function and aortic PWV (arterial stiffness) was found. The observed relationship can aid to understand the background of vascular changes in different airway diseases. Our study could be a first step to find further associations of vascular changes in different airway diseases and help guide linkage studies towards better understanding of the cardiopulmonary system.

5.3. Monozygotic twins start smoking earlier compared to dizygotic twins. Dizygotic twins smoke longer and suffer more parental smoke exposure in childhood. Monozygotic twins experience stricter smoking restrictions at home and in workplaces, but less smoke exposure in indoor public places. More monozygotic twins are ex or active smokers than dizygotics. Lesser difference exists in self-reported smoke exposure rate in monozygotic compared to dizygotic pairs concerning restaurants and cafés which is not present regarding bars, pubs and transportation facilities. Different psychological family orientation may be present across zygosity. Preventive parental care is warranted in twins families exposed to smoking.

List of publications

Peer reviewed papers with relevance to the current PhD thesis

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