What can we expect from classical twin studies? The rationale of clinical investigations based on the Hungarian twin registry

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Aims and objectives

Twin studies play a role in examining the contribution of genetic variations and environmental factors responsible for the determination of phenotypic variables and of genetic linkage between genotypes.

Monozygotic (MZ) twins share nearly 100% of their genes, since the two embryos originate from one fertilized egg. Therefore, the observed differences between the two members of a monozygotic twin pair are attributed to environmental factors. On the other hand, fraternal or dizygotic (DZ) twins share 50% of their genes, as the not at the same time born siblings, therefore, the observed differences between the members of a dizygotic twin pair are due to either genetic or environmental effects.

**Classical twin study design** involves both monozygotic and dizygotic twins. Higher correlation in monozygotic than in dizygotic twin pairs provides evidence for additive genetic influence (A) on a phenotype (effects due to genes at multiple loci or multiple alleles at one locus). Similarity of correlations suggests a contribution of the common familial environmental factors (C) shared by the twins (e.g., familiar socialization, diet, exposure to high levels of air pollution, shared womb, etc.). Finally, unique environmental factors (E), or unshared environmental factors that affect one twin but not the other, are estimated using the deviation from perfect MZ co-twin correlation (1). Univariate quantitative genetic A-C-E models are fitted to decompose phenotypic variance of the considered parameters into additive genetic effects, or heritability. The A-C-E model is able to estimate these components by capitalizing on several reasonable assumptions.

Thus, identical twins share their genome ($r=1.0$) while this correlates $r=0.5$ for fraternal twins. Moreover, on average both monozygotic and dizygotic twins equally share their common environment ($r=1$ for both monozygotic and dizygotic twins). Accordingly, environmental confounders are minimized because twin children are usually exposed to similar environments. The unique environment of co-twins remains uncorrelated for both zygosities. In the structural equation model, A-C-E components are latent variables but these variables for both co-twins are related to each other based on the described structure giving us the possibility to estimate the proportions of interest (1).

Beyond the classical ACE model, a bivariate Cholesky decomposition model can be also carried out to derive the magnitude of covariation between two investigated phenotypes of interest and to estimate what proportion of this correlation is attributable to common underlying genetic and environmental factors (2, 3). In order to estimate the amount of overlap between genes or environment that influences the two parameters, genetic ($r_g$) and environmental correlations ($r_c$, $r_e$) between a pair of measures are calculated. For
example, the genetic correlation indicates the extent to which genetic effects on one trait correlate with genetic effects on another trait, independently of the heritability of the two traits. A genetic correlation of 1.0 would indicate that genetic influences on the two traits completely overlap, whereas a genetic correlation of 0 would indicate that entirely different genes influence the two traits. By including the heritability of the measures, it is also possible to estimate the extent to which genetic and environmental factors contribute to the observed phenotypic correlation between two traits (bivariate heritability) and the extent to which the two traits share common genetic variance ($r_g^2$) (2, 3).

**Images for this section:**

![Fig. 1: Italian team (some members of the Vascular and Interventional Radiology Unit, Department of Radiological Sciences, La Sapienza University of Rome, Prof. Dr. Fabrizio Fanelli, Dr. Mario Corona, Dr. Emanuele Boatta, Dr. Chiara Zini; some members of Italian Twin Registry: Prof. Antonia Stazi, Sofia Brescianini, Emanuela Medda, Lorenza Nisticó; and members of Hungarian Twin Registry: Dr. David and Adam Tarnoki) who performed the Roman part of the International Twin Study.](image)
Methods and materials

Currently the registry consists of 500 twin pairs or multiplets (70% female, mean age 44±16 years). In the current database, we have data on risk factors, diseases and surgeries. We archived various data on past studies (eg., blood pressure, arterial stiffness; carotid, cervical and abdominal ultrasound; lung function; airway responsiveness; grip strength; body composition; echocardiography; venous distensibility and elasticity; several laboratory parameters; smoking, nutrition, physical and social activity data). Zygosity is usually assessed with multiple questions and latent class analysis in line with the recommendations of Heath et al. (5).

Results

In the past years, several imaging studies have been performed in order to understand the background of various diseases. These results are mainly presented and discussed in the order of increasing heritability estimates (Figure 2).

Assessment of non-alcoholic fatty liver disease in twins - no heritability

Nonalcoholic fatty liver disease (NAFLD) has become an exceedingly common disorder in association with the obesity epidemic, reaching a prevalence of up to 34% in developed countries. While mostly considered benign, NAFLD as a manifestation of the metabolic syndrome is an independent risk factor for cardiovascular disease. In addition, NAFLD presents as steatohepatitis in about 20% of the cases with a definite risk of progression to cirrhosis and hepatocellular carcinoma. Ongoing research aims to improve our ability of predicting disease severity and individual risk of progression.

In our study, B-mode ultrasonography was performed to detect steatosis and categorize severity in 63 monozygotic and 41 dizygotic twin pairs (age 43.7±16.7 years) (6). Limited abdominal sonography was performed by using B-mode ultrasonography (Esaote MyLab 70X Vision) equipped with a curved array transducer (1-8 MHz, CA431). Steatosis was based on increased echogenicity, loss of portal vein walls, decreased through-transmission, and closely packed echoes categorized into four groups: (i) normal liver without evidence for increased fat content and characterized by normal hepatic echogenicity and normal beam attenuation; (ii) mild steatosis, defined as the slight increase in echogenicity of liver parenchyma compared with the right kidney parenchyma and showing minimal or no decrease of visualization of hepatic vessels or their walls
and the diaphragm; (iii) moderate steatosis, characterized by the diffuse increase in liver echogenicity and slightly impaired visualization of intrahepatic vessels or their walls and the diaphragm; and (iv) severe steatosis, defined as marked increase of liver echogenicity, poor visualization of intrahepatic vessels or their walls, and increased posterior beam attenuation represented by nonvisualization of the diaphragm (7).

NAFLD was identified in 47 subjects (22.6%), of which 44 (93.6%) had mild and 3 (6.4%) had moderate steatosis. Based on 91 same-sex twin pairs, heritability analysis indicated no discernible role for genetic components in the presence of NAFLD (95% confidence interval, 0.0 to 36.0%), while shared and unshared environmental effects accounted for 74.2% and 25.8% of variations adjusted for age and BMI. These shared environmental components estimate the contribution of a common family environment of twins including factors such as diet (as the most probable factor affecting the development of NAFLD), familiar socialization, exposure to high levels of air pollution, and shared womb; whereas unshared environmental components account for factors affecting individual twins separately, such as smoking and physical activity, include measurement error, and have a moderate impact (Figure 3.).

These findings suggested a negligible role within a healthy twin population for the heritability of NAFLD as a condition defined within the limitations of sonographic criteria. The findings support the view that NAFLD and the vascular changes associated with this disorder may be first and foremost acquired through environmental effects, underscoring the importance of lifestyle in primary prevention. Radiologists should call the patient's attention to the importance of this important disorder and not disregard it.

**Carotid intima-media thickness**

As part of the International Twin Study 2009, we investigated carotid intima-media thickness (IMT) as an atherosclerotic marker in order to evaluate the influence of genetics, shared and unshared environmental components on the onset of this variable in 135 monozygotic and 70 dizygotic twin pairs (mean age 49±16 years) (8).

We demonstrated that age-adjusted intraclass correlations were higher in MZ than in DZ pairs for proximal right common carotid artery (CCA) (MZ=0.19, DZ=0.06), proximal and distal left CCA (MZ=0.27, DZ=0.06; MZ=0.27, DZ=0.13, respectively), proximal left internal carotid artery (ICA) (MZ=0.39, DZ=-0.54) suggesting a moderate genetic effect. Heritability was estimated to be 18% (95% CI: 3-33) for proximal right CCA, 26% and 27% for proximal and distal left CCA, respectively and 38% (95% CI: 26-49) for proximal left ICA. As regards distal right CCA and proximal right ICA, no genetic effects were detected (Figure 4.).
The investigated parameters appeared to be only moderately influenced by genetic factors. The moderate genetic influence on most of carotid IMT traits (18-38%) may play a role in early detection of initial atherosclerosis. Consequently, individuals with a positive family medical history of (early) cardiovascular diseases could be screened by carotid ultrasound already in young adulthood to prevent or postpone serious consequences related to increased carotid IMT. Furthermore, increased carotid IMT could be prevented or postponed if the underlying unshared environmental factors, which are largely responsible for these traits, could be appropriately managed in high-risk patients (8).

**Intima-media thickness of femoral arteries and internal jugular vein**

Peripheral artery disease is a common atherosclerotic disorder related to serious complications. As part of the still ongoing GLOBAL twin study, our aim was to assess the heritable affects on the ultrasonographically determined *intima-media thickness of common, deep and superficial femoral arteries* (CFA, DFA, SFA) which is frequently used as an indicator of generalized and coronary atherosclerosis.

52 Hungarian (26 monozygotic and 26 dizygotic) twin pairs (mean age 56±8 years) underwent B-mode sonography of bilateral CFA, SFA, and origin of DFA. Wall thickness was measured automatically by QLAB software. All phenotypic variables were screened for outliers and normality. Concordance between MZ and DZ pairs were assessed by Pearson correlations. Rough heritability was calculated according to the Falconer's formula, since ACE model could not be performed due to the relatively low number of twin pairs.

Mean IMT values of left and right CFA, DFA and SFA indicated 0.6±0.1 mm, 0.6±0.2 mm, 0.5±0.1 mm, 0.6±0.1 mm, 0.5±0.1 mm and 0.5±0.1, respectively. A high-moderate heritability was found in case of right and left CFA (1.0 and 0.52) and SFA (0.38 and 0.67), while DFA showed a weak genetic dependency (0.33 and 0.09, respectively) (*Figure 5*).

Most of the femoral artery IMT values are mainly influenced by the environmental factors which underscores the importance of lifestyle in the increase of femoral IMT values. Radiologist should call the patient's attention to the prevention of modifiable risk factors in case of early femoral atherosclerosis. In order to quantify the relative influence of additive genetic, common environmental and unique environmental effects on the twin pairs further investigation is warranted.

As part of the same study, we also measured the **wall thickness of internal jugular vein (IJV)** since multiple sclerosis was hypothesized to be associated with thickened wall of IJV. We measured wall thickness automatically by QLAB software.
Mean right and left IJV wall thickness indicated 0.63±0.13 mm and 0.59±0.12 mm, respectively. A substantially moderate genetic dependency was found on right IJV wall thickness (0.55), while the heritability was lower on the left side (0.09).

Accordingly, wall thickness of the internal jugular vein seemed to be moderately-poorly heritable. This finding might stimulate further research on venous disorders related to IJV wall pathology and help to predict the success of venous angioplasty.

**Imaging studies in twins with COPD**

Current understanding of the pathogenesis of chronic obstructive pulmonary disease (COPD) suggests that chronic inflammation due to tobacco smoking and occupational dust exposure leads to the airways obstruction and parenchymal destruction (9). However, only a minority of exposed individuals develops COPD, and some individuals may be more susceptible to exogenous factors, and that the susceptibility to the development of this condition could be genetically determined (9). Our aim was to investigate whether specific lung alterations detected at chest computed tomography (CT) in twin pairs may suggest peculiar genetic aspects in COPD.

We conducted a search among 430 twin pairs in the Hungarian Twin Registry for twin pairs with the anamnesis of COPD. Five Hungarian twin pairs (3 monozygotic and 2 dizygotic) were found and recruited for a low-dose high resolution computer tomography (HRCT) which was performed in inspiration and expiration (Philips Brilliance 16, Philips Healthcare, Amsterdam, The Netherlands) at least 15 minutes after the inhalation of 400 µg salbutamol in order to better visualize the presence of thickened wall bronchi, bronchial dilatation or bronchial obstruction. Multiplanar reconstructions were used in order to assess the presence of thickened wall bronchi, atelectasies and mucus plugs (10). Automatic 3D volume reconstruction (Philips Healthcare, Amsterdam, The Netherlands) was performed from the acquired images to assess the lung volume in inspiration and expiration. 2 mm axial series were reconstructed from the 3 mm slices for density analysis using automatic software (Lung Emphysema, Philips Healthcare, Amsterdam, The Netherlands) which calculated the mean lung density, emphysema and air trapping percentages for total, right and left lung. On the inspiratory scans, the extent and severity of emphysema was estimated using standardized threshold cut-off analysis where the per cent of voxels with attenuation less than -950 Hounsfield Units (HU) was considered to be emphysema as recommended by the COPD Foundation (10). The extent of gas trapping was estimated on the expiratory CT scans using the same automatic software and a threshold value of -850 HU, and the percentage of the lung beyond this point (hyper inflated areas) was calculated (10). A greater visual similarity of findings in MZ than DZ twins suggested a genetic influence, while equal or lesser similarity suggests the role of environmental factors.
In general, we found greater visual similarities in some COPD features (lung density, presence of bronchial wall thickening, bronchiectasis and/or mucus plug formation, air trapping and emphysema score) in MZ twins compared to DZ twins indicating a genetic heredity. Monozygotic twins were more similar (average difference was 382 cm$^3$) in the lung volume expiration capacity compared to DZ twins (2303 cm$^3$). Volume reconstruction images also showed remarkable similarities in the volume patterns of MZ twins. The average difference in air trapping score was smaller in MZ twins compared to DZ twins (17.6% vs. 26.6%) regardless of the history of COPD (Figures 6 and 7).

These findings, if validated by other laboratories, should stimulate the search for genes responsible for these traits and draw the attention of the radiologists to the possible familial transmission of these CT features in subjects with COPD and might improve our understanding of the morphologic expression of COPD (11).

**Assessment of femoral vein capacity and elasticity of twins**

Chronic venous disease is a common cardiovascular disorder in Northern and Western Europe, however, the etiologic factors predisposing individuals to the disease are unclear. The altered venous biomechanics may contribute to the pathogenesis of venous diseases.

102 (78 monozygotic and 24 dizygotic) twin pairs (mean age 50.5±16.1 years) underwent measurements of anteroposterior and mediolateral diameters of the common femoral vein by ultrasonography both in supine and standing body positions, with or without controlled forced expiration (i.e. Valsalva test).

We demonstrated a 39.3% heritability of the variance of low pressure, 37.9% of high pressure venous capacity and 36.4% of maximal capacity changes even after adjustment for age, sex and body weight. This value in the standing body position could reach 50%. Bivariate Cholesky analysis revealed substantial covariance of inherited body weight and venous capacity components (57-81%).

Altered venous capacity may be a predisposing phenotypic factor for the development of chronic venous disease, and venous capacity may be altered before the onset of the symptoms of chronic venous disease. The substantial genetic influence of venous capacity found in our results may highlight the importance of early screening, detection and prevention of related venous diseases in high-risk patients. Elucidation of the genes that influence venous biomechanics at different pressures and in different body positions may provide in the future important new insight into the pathophysiology of venous
diseases that are associated with altered venous biomechanics. Our work might yield some new insight into the inheritance of venous diseases that are associated with altered venous biomechanics and help elucidation of the involved genes (12).

**Inheritance of carotid plaque characteristics**

Carotid artery plaques have been associated with multiple complications such as cardiovascular events, retinal or cerebral ischemia, and all-cause mortality. Few family studies reported moderate genetic impact on the presence and scores of carotid plaques (13-15). However, the heritability of carotid plaque characteristics remained still unclear.

In this study, 192 monozygotic and 83 dizygotic adult twin pairs (age 49±15 years) from Hungary, Italy and the United States underwent B-mode and Color-Doppler ultrasound of bilateral common, internal and external carotid arteries with linear array high frequency (5-10 MHz) transducers (in Rome: Esaote Technos MPX, in Padua: Philips iU22, in Perugia: Esaote Technos MP, in Hungary: Toshiba Power Vision and Esaote MyLab70, in the USA: Sonosite Titan). Carotid plaque was defined as an endoluminal protrusion of at least 1.5 mm or a focal thickening >50% of the intima-media thickness relative to the adjacent wall segment (16). The investigators assessed bilaterally the presence, sidedness, number of carotid plaques and their size in mm² on common carotid artery, proximal internal carotid artery and external carotid artery (ECA).

Age-, sex- and country-adjusted heritability was 78% for the presence of carotid plaque (95% confidence interval /CI/, 55 to 90%), 74% for plaque echogenicity (hypoechoic, hyperechoic or mixed; 95% CI, 38 to 87%), 69% for plaque size (area in mm² in longitudinal plane; under or over 50 percentile) (95% CI, 16 to 86%), 74% for plaque sidedness (unilateral or bilateral; 95% CI, 25 to 90%), 74% for plaque numerosity (95% CI, 26 to 86%), 68% (95% CI, 40 to 84) and 66% (95% CI, 32 to 90) for the presence of plaque in carotid bulbs and proximal internal carotid arteries. No role of shared environmental factors was found. Unique environmental factors were responsible for the remaining variance (22-34%) (Figure 8.).

Accordingly, the heritability of ultrasound characteristics of carotid plaque was high. Unshared environmental effects account for a modest portion of the variance. Carotid ultrasound might represent a very useful screening method in individuals with a family history of early cardio- and cerebrovascular events. Furthermore, our findings should stimulate the search for genes responsible for these traits. On the other hand, our study shows that environmental contribution has a moderate role in preventing, delaying or attenuating carotid plaque formation. The "traditional" concept of the deterministic role of
individual-specific modifiable environmental factors, such as smoking, unhealthy nutrition or reduced physical activity still remains important (17).

Images for this section:

Fig. 2: Heritability estimates of investigated variables in increasing order. Variables of COPD twin study are not displayed due to the limited number of subjects and lack of heritability calculation. *Rough heritability estimates (unadjusted values). R: right, L: left, CFA: common femoral artery, IMT: intima-media thickness, SFA: superficial femoral artery, IJV: internal jugular vein, ICA: internal carotid artery, DFA: deep femoral artery, NAFLD: non-alcoholic fatty liver disease
**Fig. 3:** Grey scale ultrasound image of a 40 year old monozygotic female twin pair. A characterizes the first-born, while B means the second-born twin. Twin A showed signs of hyperreflective liver, whereas her twin sister (twin B) did not, which suggests the role of environmental effects in the development of non-alcoholic fatty liver disease.

**Fig. 4:** Heritability (A), common (C) and unique (E) environmental factors of bilateral proximal and distal common carotid artery (CCA) and proximal internal carotid artery (ICA). Variances and 95% confidence intervals are shown.
Fig. 5: Automatic Intima-media thickness (IMT) measurement (QLAB) on left (L) and right (R) common femoral artery (CFA) in Hungary’s only twin pair who were separated at birth, reared apart and met approximately 50 years later. The female twin pair is 57 years old and IMT values show remarkable similarities despite of the different environment where the twins grew up, indicating a possible genetic influence. Note the hypoechoic plaques on both right common femoral arteries. A characterizes the first-born, while B means the second-born twin.
**Fig. 6:** Distribution of air trapping in a 50 year old male monozygotic twin pair, colored MPR image (expiratory scan, coronal view). Note the similar distribution in location and degree. A characterizes the first-born, while B means the second-born twin. Red color represents the distribution of the per cent of voxels with attenuation less than -850 HU.

**Fig. 7:** Distribution of emphysema in a 64 year old female monozygotic twin pair, colored axial HRCT image. A characterizes the first-born, while B means the second-born twin.
Blue color represents the distribution of the per cent of voxels with attenuation less than -950 HU. The second-born (B) twin developed COPD (GOLD stage III.) had smoked for longer (6 years) than her twin sister (A) who had no COPD yet, but increased number of emphysematous areas can be already seen.

Fig. 8: Identical localisation and size of carotid plaques on the proximal portion of right internal carotid arteries in a 64-year-old Italian female twin pair, suggesting the role of genetic effects in the development of carotid plaques. A characterizes the first-born, while B means the second-born twin.
Conclusion

Hungarian Twin Registry was founded in 2007 including over 500 twin pairs, of which a maximum of 160 twin pairs attended (mainly ultrasound) imaging studies which contributed to the current understanding on the background of several disorders. Among other phenotypes, carotid and femoral intima-media thickness, jugular vein wall thickness have been also assessed by ultrasound. Genetic factors have been suspected on the development of radiological lung parenchymal and small airway changes in chronic obstructive pulmonary disease on high resolution computed tomography scans of monozygotic twin pairs. Moderate heritability of femoral vein capacity and elasticity was demonstrated by ultrasound (30-50%). Absence of genetic background in non-alcoholic fatty liver disease (0%) and high inheritance of carotid plaque characteristics (66-78% for the presence of carotid plaque, plaque echogenicity, size, sidedness numerosity and presence of plaque in carotid bulbs and proximal internal carotid arteries) were demonstrated.

Hungarian Twin Registry is open to any kind of collaboration, study ideas are welcome.

Personal information

Adam Domonkos Tarnoki, MD, PhD
David Laszlo Tarnoki, MD, PhD
Department of Radiology and Oncotherapy, Semmelweis University, Budapest
Address: 78/Aüllőistreet, 1082, Budapest, Hungary
Tel.: +36-30-368-7843
Fax: +36-1-2780367
Email: tarnoki2@gmail.com, tarnoki4@gmail.com

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