Twins Lead to the Prevention of Atherosclerosis: Preliminary Findings of International Twin Study 2009

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ABSTRACT

Introduction.—Atherosclerosis is an inflammatory process in which the artery wall thickens as a result of plaque deposition, but this process may be preceded by increased arterial stiffness. We sought to evaluate the influence of genetics and shared and unshared environmental components on the onset of atherosclerosis.

Methods.—A total of 135 monozygotic (MZ) and 70 dizygotic (DZ) twin pairs (mean age 49 ± 16 years) underwent carotid intima media thickness (IMT; carotid analyzer) and arterial stiffness (augmentation index on brachial artery [Aix\textsubscript{bra}], pulse wave velocity on aorta [PWV\textsubscript{ao}]; TensioMed Arteriograph) measurements.

Results.—Age-adjusted intraclass correlations were greater 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), and proximal left internal carotid artery (ICA; MZ = 0.39, DZ = −0.54), suggesting a moderate genetic effect. Heritability was estimated to be 18% (95% confidence interval [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. Regarding distal right CCA and proximal right ICA, no genetic effects were detected. Age-adjusted intraclass correlation of Aix\textsubscript{bra} and PWV\textsubscript{ao} were 0.65 (95% CI = 0.55–0.72) and 0.46 (95% CI = 0.33–0.57) in MZ, 0.42 (95% CI = 0.24–0.57) and 0.28 (95% CI = 0.08–0.47) in DZ pairs; heritability 45% (95% CI = 12–71%) and 42% (95% CI = 2–57%) adjusted by age, respectively.

Conclusions.—The investigated parameters appeared to be only moderately influenced by genetic factors. Environmental factors of relevance for these measures appeared not to be shared within family but related to individual experience (e.g., smoking habits, diet, and physical activity). Atherosclerosis detection at an early stage is necessary for treatment to prevent serious complications such as stroke and heart attack.

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Introduction

Atherosclerosis is an inflammatory process and a multifactorial metabolic disorder in which the artery wall thickens as a result of plaque deposition, but this process may be preceded by increased arterial stiffness. Atherosclerosis underlies various clinical cardiovascular diseases that are known to aggregate within families, such as coronary heart disease, myocardial infarction, and stroke.\(^1\) Although there is a consensus that genetic factors play a role in atherogenesis,\(^6\) the precise magnitude of the genetic influence is poorly described.

Carotid intima-media thickness (IMT), or the thickness of the first two layers (intima and media) of the carotid artery, is a surrogate marker for atherosclerosis and is associated with prevalent and incident cardiovascular disease.\(^7\)–\(^10\) The authors of recent studies\(^11\)–\(^13\) have shown that carotid IMT is mostly determined by genetic factors. However, the precise extent to which genetic predisposition explains the variance of carotid IMT is unclear. In addition, it is not clear what connection is between the carotid IMT and arterial stiffness measures. Heritability estimates of carotid IMT range from 0.24 to 0.59 on the basis of three twin studies reported recently.\(^13\)–\(^15\) Arterial stiffness is a dynamic property that is determined by vascular function such as vascular smooth muscle tone and by the structure of the vessel wall such as elastin/collagen content. Regional arterial stiffness is measured indirectly by measuring pulse wave velocity over the arterial segment. Pulse wave velocity on aorta (PWV\(\text{ao}\)) is widely recognized as a direct marker of arterial stiffness\(^16\),\(^17\) and inversely related to arterial distensibility. It is calculated by the following formula:

\[
PWV_{\text{ao}} = \text{distance (m)/transit time (sec)}.\]

PWV\(\text{ao}\) is correlated with cardiovascular risk factors,\(^18\) and it is a strong vascular risk factor for prediction of mortality in elderly subjects\(^19\) and in the general population.\(^20\) Dividing the augmentation pressure (difference between the second and first systolic peaks, P\(_2\)−P\(_1\)) by the pulse pressure gives the augmentation index (Aix\(\text{brach})), which is the indirect marker of endothelial dysfunction and being used ever more often in studies as parameters of wave reflection.\(^21\),\(^22\)

Heritability of augmentation index in women has been investigated by some authors,\(^23\) who reported a 37% genetic component for this measure. Most of the studies relate to invasively measured arterial stiffness. Recent studies indicate that the Aix\(\text{brach}\) can be measured accurately noninvasively, providing a reliable method of studying arterial properties in a large population as screening method.\(^24\)

With the applanation tonometry (SphygmoCor; PWV Medical, Sydney, Australia), both PWV\(\text{ao}\) and Aix\(\text{brach}\) can be measured noninvasively. The piezo-electronic method (Compilor; ALAM Medical, Smyrna, GA) records pulse waves via pressure transducers and determines the PWV\(\text{ao}\) but is not able to measure the augmentation. A clinically validated investigator-independent oscillometric method\(^25\) for determining arterial stiffness was used in our study. The heritability of carotid intima media thickness also was investigated. The goal of this investigation was to assess the phenotypic correlations between carotid IMT and arterial stiffness measures using the same twin sample.

Methods

Subjects

A total of 205 twin pairs (70 dizygotic [DZ] and 135 monozygotic [MZ] twin pairs, mean age 49 ± 16 years ± SD) were recruited in this classical twin study, including 44 Hungarian (10 DZ and 34 MZ, mean age 44 ± 16.1 years ± SD, min. 18, max. 82 years), 124 Italian (58 DZ and 66 MZ, mean age 58 ± 9 years ± SD, min. 23, max. 73 years), and 37 American (2 DZ and 35 MZ, mean age 47 ± 17.4 years ± SD, min. 17, max. 77 years) pairs (see Table 1 for subject characteristics).

Subjects were recruited as part of the International Twin Study 2009 project. A random sample of twins older than 18 years of age living in Hungary, the United States, and Italy was invited to participate. Hungarian twins were measured during two Hungarian twin festivals (Szigethalom, Agfalva) in July 2009 or at two large hospitals in Budapest between July and September 2009. American twins were recruited during the Twins Days Festival in Twinsburg, Ohio, in August 2009. Italian twins were enrolled in Rome and Padua by the Italian Twin Registry\(^26\) in September 2009. Exclusion criteria were pregnancy, medical conditions possibly interfering with compliance during test procedures, and acute infection within 3 weeks of measurement. All subjects were asked not to smoke for 3 hours, not to eat 1 hour, not to drink alcohol and coffee 10 hours before their visit. The presence of risk factors, medication, and clinical symptoms were recorded by the attending physicians.

Lacking genotyping of the sample and to maximize the accuracy of zygosity classification, we used a multiple self-reported question approach to assess zygosity. The most likely zygosity was assigned on the basis of the seven self-reported responses.\(^27\)

First, height was measured in the spot and weight was measured by OMRON BF500 body consistency monitor device. These measures were used to calculate body mass index (BMI) as weight in kilograms divided by height in squared meters. Then systolic blood pressure and diastolic blood pressure were measured by TensioMed Arteriograph (TensioMed, Budapest, Hungary) on the dominant arm with the subject in supine position after 10 minutes of rest. A medical history and a physical examination were obtained from all twin subjects. Physical activity and smoking habits were assessed by means of a questionnaire documenting level and duration of past and present physical activity, including sport and nonsports activities. Cigarette smoking was classified into current smoker versus never or ex smoker. Pack-years of smoking were calculated as the number of packs of cigarettes smoked per day times the number of years smoked. Finally, carotid ultrasound was performed.
### Table 1

*Clinical Characteristics and Measures According to Gender and Country*

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>Hungarian</th>
<th>Italian</th>
<th>American</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, n</td>
<td>136</td>
<td>274</td>
<td>88</td>
<td>248</td>
<td>74</td>
<td>410</td>
</tr>
<tr>
<td>Zygosity, MZ:DZ</td>
<td>97:39*</td>
<td>186:88*</td>
<td>34:10†</td>
<td>66:58‡</td>
<td>35:2‡</td>
<td>135:70‡</td>
</tr>
<tr>
<td>Mean age, years</td>
<td>49 ± 18 (46–52)</td>
<td>49 ± 16 (47–51)</td>
<td>44 ± 16.1 (40.6–47.4)</td>
<td>58 ± 9 (56.8–59.1)</td>
<td>47 ± 17.4 (43.0–50.9)</td>
<td>49 ± 16.4 (47.4–50.6)</td>
</tr>
<tr>
<td>Male, % (95% CI)</td>
<td>—</td>
<td>—</td>
<td>31 (21.3–40.6)</td>
<td>36 (30.0–42.0)</td>
<td>16 (7.6–24.3)</td>
<td>31 (26.5–35.4)</td>
</tr>
<tr>
<td>Female, % (95% CI)</td>
<td>—</td>
<td>—</td>
<td>69 (59.3–78.6)</td>
<td>64 (58.0–70.0)</td>
<td>84 (75.6–92.3)</td>
<td>69 (64.5–73.5)</td>
</tr>
<tr>
<td>BMI, kg/m² (mean ± SD) (95% CI)</td>
<td>26.6 ± 3.2 (26.1–27.1)</td>
<td>26.8 ± 3.8 (26.3–27.2)</td>
<td>24.6 ± 4.5 (23.6–25.5)</td>
<td>26.6 ± 4.4 (26.1–27.1)</td>
<td>27.4 ± 6.3 (25.9–28.8)</td>
<td>26.3 ± 4.8 (25.8–26.7)</td>
</tr>
<tr>
<td>Never smokers, % (95% CI)</td>
<td>59.6 (50.7–67.2)</td>
<td>60.7 (55.2–67.8)</td>
<td>73.9 (64.8–83.1)</td>
<td>41.4 (34.9–47.1)</td>
<td>70.2 (59.5–80.4)</td>
<td>60.5 (55.2–64.7)</td>
</tr>
<tr>
<td>Ex smokers, % (95% CI)</td>
<td>30.6 (22.3–37.7)</td>
<td>26.9 (21.7–32.2)</td>
<td>10.9 (4.4–17.5)</td>
<td>39.4 (32.9–45.1)</td>
<td>28.7 (18.6–39.3)</td>
<td>27.8 (23.6–32.3)</td>
</tr>
<tr>
<td>Current smokers, % (95% CI)</td>
<td>9.6 (4.6–14.5)</td>
<td>12.2 (8.1–15.8)</td>
<td>15.1 (7.5–22.5)</td>
<td>19.1 (14.1–23.9)</td>
<td>1.1 (0–3.48)</td>
<td>11.6 (8.8–15.1)</td>
</tr>
<tr>
<td>SBP, mmHg (mean ± SD) (95% CI)</td>
<td>131.4 ± 16.1 (128.7–134.1)</td>
<td>127.9 ± 10.5 (126.6–129.1)</td>
<td>127.9 ± 16.6 (124.4–131.4)</td>
<td>130.6 ± 18.6 (128.3–132.9)</td>
<td>126.2 ± 16.8 (122.3–130.0)</td>
<td>128.8 ± 17.5 (127.1–130.5)</td>
</tr>
<tr>
<td>DBP, mmHg (mean ± SD) (95% CI)</td>
<td>79.7 ± 11.6 (77.7–81.6)</td>
<td>75.5 ± 18.8 (73.2–77.7)</td>
<td>75.2 ± 11.2 (72.8–77.5)</td>
<td>78.8 ± 10.4 (77.5–80.1)</td>
<td>71.5 ± 9.6 (69.3–73.7)</td>
<td>76.1 ± 10.9 (75.0–77.1)</td>
</tr>
</tbody>
</table>

BMI, body mass index; 95% CI, 95% confidence interval; DBP, diastolic blood pressure; DZ, dizygotic; MZ, monozygotic; SBP, systolic blood pressure; SD, standard deviation.

*Number of subjects.
†Number of twin pairs.
Measurement of Carotid IMT

All participants underwent the measurement of carotid ultrasound via B-mode ultrasound (in Rome, Italy: Esaote Technos MPX [Biosound Esaote, Indianapolis, IN]; in Hungary: Toshiba Power Vision [Toshiba, Toyko, Japan] and Esaote Mylab40; and in the United States: Sonosite Titan [Bothell, WA]). Linear array transducers were used (7.5 MHz in Rome, 5–10 MHz in Hungary, and the L38 linear array transducer 4–7 MHz in the United States). In Padua we performed the IMT study with a high-resolution color-coded duplex sonography scanner (Philips iU22) using a high-frequency (5-10 MHz) linear probe and with a dedicated software (QLAB) that measures automatically the IMT thickness.

IMT of proximal and distal CCAs and of the proximal ICA was measured bilaterally with the use of high-resolution B-mode ultrasonography with standard techniques.9,28 IMT was quantified on the far wall of the CCA 3-5 cm after its origin from subclavian artery (proximal CCA) and 1 cm proximally to the bifurcation (distal CCA). In addition, IMT was measured on the far wall at the proximal left and right ICA 1 cm distal to the bifurcation. For each segment, the sonographer used multiple different scanning angles to identify the longitudinal image of IMT showing the maximum IMT. At least 10 pictures for each segment were stored digitally, and measurements were made off-line by the use of semi-automated computerized analytical software (Carotid Analyzer; Medical Imaging Applications LLC, Coralville, IA). Average values of the IMT of each of the six measurement spots (both proximal CCA, distal CCA, proximal ICA) were used as the IMT values for each twin in the analysis. We did not use electrocardiogram gating. In case of a carotid plaque, we measured the carotid IMT at the end of the plaque. Carotid ultrasound tests were conducted by the local physicians and the authors.

Arterial Stiffness Measurement

Arterial stiffness measures (Aix\textsubscript{bra}, PWV\textsubscript{ao}) were assessed via use of the TensioMed Arteriograph. The measurements were performed in accordance with guidelines recommended by the European Society of Cardiology.29 Subjects were asked to hold the regulations before the test. In addition, they were tested in supine position on the dominant arm after at least 10 min of rest. Subjects were asked not to speak and move during the measurements with closed eyes.

All arterial stiffness tests were performed by the two authors (ADT and DLT) in each research places and by the same device. Carotid ultrasound and arterial stiffness measurements were conducted at the same time on both members of the twin pair.

Statistical Analysis

Risk Factor Assessment. Initially we conducted a descriptive analysis (mean, SD, and the percentage for categorical variables) for all risk factors, the arterial stiffness (Aix\textsubscript{bra} and PWV\textsubscript{ao}) and the carotid IMT values in MZ and DZ twins. Intercountry differences of the carotid IMT parameters were calculated by the use of the Student’s t-test. Differences were considered as significant for \( p < 0.05 \).

Estimating Genetic Influence on Arterial Stiffness and Carotid IMT. A descriptive estimate of the genetic influence on arterial stiffness and carotid IMT were calculated by use of the intraclass correlation in MZ (rMZ) and DZ (rDZ) pairs. The corresponding 95% confidence intervals for rMZ and rDZ were calculated.30 If the within pair similarity for a phenotype, such as Aix\textsubscript{bra}, PWV\textsubscript{ao}, and carotid IMT, is greater in MZ than DZ pairs this provides evidence for genetic influence.

Structural equation modeling was used to estimate heritability. Univariate quantitative genetic model was performed to decompose phenotypic variance of the considered parameters into additive (A), nonadditive (D), common environmental (C), and unique environmental (E) effects. The additive genetic component measures the effects caused by genes at multiple loci or multiple alleles at one locus. The nonadditive or dominant component measures the interaction between alleles at the same locus or on different loci. The common environmental component estimates the contribution of the shared family environment by both twins, whereas the unique environmental component estimates the effects that apply only to each individual twin, and includes measurement error. Model fitting was done with the statistical software MX (Michael C. Neale, Richmond, VA),31 and all the analyses were adjusted by age.

The fitting model was determined on intraclass correlation: if twin correlations do not suggest shared environmental influence the (A+D) E model was considered (Broad heritability); if twin correlations show evidence of common environmental effects the full ACE model was considered. All classic twin studies, including the current study, are based on the equal environments assumption (MZ and DZ twins are similarly exposed to the same shared environment). Correlation coefficients (CC) between Aix\textsubscript{bra} or PWV\textsubscript{ao} and carotid IMT were calculated to measure the strength and the direction of a linear relationship between variables, furthermore scatter plots were used to show graphically the relationships between parameters.

Results

Clinical Characteristics and Measures

Table 1 presents clinical characteristics of the sample by sex and countries. A total of 44 Hungarian, 124 Italian, and 37 American twin pairs were included in the analysis. A total of 66.4% of all twins were monozygotic, and 33.6% were dizygotic. Females comprised 66.8% of the study population. Both sexes were comparable with respect to age, BMI, smoking habits, race, and ethnicities.

Italian twin pairs were older than the other countries’ samples. The prevalence of never smokers was greater in Hungarian and American sample compared with the Italians. Ex-smokers’ prevalence were significantly lower in the Hungarian sample compared with the others. The prevalence of American current smoker twins was very low. Never smokers’ rate was similar in
Table 2

Mean, SD Values and Confidence Intervals of the Investigated Carotid Intima Media Thickness Parameters According to Sample Countries

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hungarian</th>
<th>American</th>
<th>Italian</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right CCA proximal IMT, mean ± SD (95% CI)</td>
<td>0.62 ± 0.16* (0.59–0.66)</td>
<td>0.60 ± 0.14† (0.57–0.64)</td>
<td>0.67 ± 0.14 (0.65–0.68)</td>
<td>0.65 ± 0.14 (0.63–0.66)</td>
</tr>
<tr>
<td>Right CCA distal IMT, mean ± SD (95% CI)</td>
<td>0.65 ± 0.34 (0.58–0.72)</td>
<td>0.66 ± 0.16‡ (0.62–0.69)</td>
<td>0.70 ± 0.17 (0.68–0.73)</td>
<td>0.69 ± 0.22 (0.66–0.70)</td>
</tr>
<tr>
<td>Right ICA proximal IMT, mean ± SD (95% CI)</td>
<td>0.70 ± 0.37 (0.62–0.78)</td>
<td>0.69 ± 0.16 (0.65–0.73)</td>
<td>0.68 ± 0.17 (0.66–0.71)</td>
<td>0.69 ± 0.23 (0.66–0.71)</td>
</tr>
<tr>
<td>Left CCA proximal IMT, mean ± SD (95% CI)</td>
<td>0.62 ± 0.20§ (0.58–0.66)</td>
<td>0.62 ± 0.14 (0.58–0.65)</td>
<td>0.70 ± 0.15 (0.68–0.72)</td>
<td>0.67 ± 0.16 (0.65–0.68)</td>
</tr>
<tr>
<td>Left CCA distal IMT, mean ± SD (95% CI)</td>
<td>0.65 ± 0.27§ (0.60–0.71)</td>
<td>0.62 ± 0.15† (0.58–0.65)</td>
<td>0.73 ± 0.20 (0.71–0.76)</td>
<td>0.69 ± 0.21 (0.67–0.71)</td>
</tr>
<tr>
<td>Left ICA proximal IMT, mean ± SD (95% CI)</td>
<td>0.66 ± 0.42 (0.57–0.74)</td>
<td>0.71 ± 0.20 (0.66–0.76)</td>
<td>0.69 ± 0.19 (0.67–0.71)</td>
<td>0.69 ± 0.25 (0.66–0.71)</td>
</tr>
</tbody>
</table>

CCA, common carotid artery; 95% CI, 95% confidence interval; ICA, internal carotid artery; IMT, intima media thickness; SD, standard deviation.

*Hungarian vs Italian \( p = 0.02 \).
†American vs Italian \( p < 0.01 \).
‡American vs Italian \( p = 0.04 \).
§Hungarian vs Italian \( p < 0.01 \).

Both genders. Physical activity, total cholesterol, total triglycerides, and blood glucose were not analyzed.

Statistically significant differences across countries, as indicated by confidence intervals and \( p \) values, were observed for some IMT measurements (Table 2). \( A_{\text{ix}} \text{bra} \) and \( PWV_{ao} \) mean values were as follows: Hungary: −26.4% (95% CI = −31.6, −21.7) and 8.8 m/sec (95% CI = 8.4, 8.4), United States: −29.5% (95% CI = −39.5, −24.2), and 8.7 m/sec (95% CI = 8.2, 9.2), Italy: −4.4% (95% CI = −7.9, −0.8) and 9.3 m/sec (95% CI = 9.0, 9.6), respectively.

Genetic and Environmental Effects on Arterial Stiffness and Carotid IMT

Table 3 presents the age-adjusted intraclass correlation of IMT values by zygosity. Age-adjusted intraclass correlations were greater in MZ than in DZ pairs for proximal right 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 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.

The age-adjusted intraclass correlation of \( A_{\text{ix}} \text{bra} \) and \( PWV_{ao} \) were 0.65 (95% CI = 0.55–0.72) and 0.46 (95% CI = 0.33–0.57) in MZ, 0.42 (95% CI = 0.24–0.57) and 0.28 (95% CI = 0.08–0.47) in DZ pairs; indicating heritability of 45% (95% CI = 12–71%) and 42% (95% CI = 2–57%) adjusted by age, respectively (Table 4).
Table 5 and Figure 1 show the results of the analysis aimed to estimate phenotypic correlation coefficients between the \( A_{\text{ix}_{\text{bra}}} \), \( PWV_{\text{ao}} \), and carotid IMT parameters. Overall the estimated coefficients indicated a low, not significant linear correlation, and few slight differences in coefficient values between countries were observed. Figures 2, 3, and 4 show the sample ultrasound images where the arterial stiffness was optimal, increased and abnormal.

### Discussion

Twin studies are one of a family of designs in genetics that aid the study of individual differences by highlighting the role of environmental and genetic causes on diseases. Twins are invaluable for studying these important questions because they disentangle the sharing of genes and environments. The twin design compares the similarity of identical twins who share 100% of their genes, to that of dizygotic or fraternal twins, who share on average 50% of their genes. This is the first study of twins to investigate the relative contribution of genetic and environmental influence on carotid IMT and arterial stiffness together in a large international twin sample conducted by international multi-disciplinary researchers and physicians. Our results indicate that the investigated measures of arterial stiffness (\( A_{\text{ix}_{\text{bra}}} \) and \( PWV_{\text{ao}} \)), and most of the carotid IMT parameters appeared to be only moderately influenced by genetic factors and mostly influenced by environmental factors. These environmental factors of relevance for these measures appeared not to be shared within families but were related to the individual (e.g., smoking habits, diet, and physical activity). Former studies conducted on only male or only female subjects indicated that additive genetic influences explain a significant proportion of the interindividual variation in carotid IMT and results similar to ours were found for arterial stiffness values.

Our heritability estimates of carotid IMT are partly consistent with previous findings. Most studies reported that genetic factors account for 0.24–0.59 of carotid IMT variation in families after adjustment for traditional cardiovascular risk factors, but we found lower values in our international sample. A very high heritability estimate of carotid IMT (0.92) was reported in a Mexico population, but the sample size of the study was very small. Swan et al. estimated the heritability of carotid IMT of 0.31 in a Scottish twin sample. A Finnish study reported a modest heritability

### Table 4

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Zygosity</th>
<th>Intraclass Correlation (age adjusted)</th>
<th>95% CI</th>
<th>A (95% CI)</th>
<th>C (95% CI)</th>
<th>E (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( A_{\text{ix}_{\text{bra}}} )</td>
<td>MZ</td>
<td>0.65</td>
<td>0.55–0.72</td>
<td>0.45 (0.12–0.71)</td>
<td>0.20 (0.00–0.49)</td>
<td>0.35 (0.28–0.45)</td>
</tr>
<tr>
<td></td>
<td>DZ</td>
<td>0.42</td>
<td>0.24–0.57</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( PWV_{\text{ao}} )</td>
<td>MZ</td>
<td>0.46</td>
<td>0.33–0.57</td>
<td>0.42 (0.02–0.57)</td>
<td>0.04 (0.00–0.3)</td>
<td>0.54 (0.43–0.67)</td>
</tr>
<tr>
<td></td>
<td>DZ</td>
<td>0.28</td>
<td>0.08–0.47</td>
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</tbody>
</table>

A, additive genetic variance; \( A_{\text{ix}_{\text{bra}}} \), augmentation index on brachial artery; DZ, dizygotic; C, shared environmental variance; CCA, common carotid artery; 95% CI, 95% confidence interval; E, unique environmental variance; MZ, monozygotic; \( PWV_{\text{ao}} \), pulse wave velocity on aorta.

### Table 5

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No. Twin Subjects</th>
<th>( A_{\text{ix}_{\text{bra}}} )</th>
<th>( PWV_{\text{ao}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Partial CC (Age Adjusted)</td>
<td>Pearson CC (Nonage Adjusted)</td>
</tr>
<tr>
<td>Right CCA proximal IMT</td>
<td>386</td>
<td>0.0241 ( ( p = 0.63 ) )</td>
<td>0.3207 -0.027 ( ( p = 0.59 ) )</td>
</tr>
<tr>
<td>Right CCA distal IMT</td>
<td>379</td>
<td>0.0888 ( ( p = 0.08 ) )</td>
<td>0.3284 0.0669 ( ( p = 0.19 ) )</td>
</tr>
<tr>
<td>Right ICA proximal IMT</td>
<td>384</td>
<td>0.0044 ( ( p = 0.93 ) )</td>
<td>0.1823 0.0964 ( ( p = 0.06 ) )</td>
</tr>
<tr>
<td>Left CCA proximal IMT</td>
<td>384</td>
<td>0.1027 ( ( p = 0.04 ) )</td>
<td>0.3822 0.0136 ( ( p = 0.79 ) )</td>
</tr>
<tr>
<td>Left CCA distal IMT</td>
<td>384</td>
<td>0.0812 ( ( p = 0.11 ) )</td>
<td>0.3816 0.0307 ( ( p = 0.54 ) )</td>
</tr>
<tr>
<td>Left ICA proximal IMT</td>
<td>383</td>
<td>-0.0153 ( ( p = 0.76 ) )</td>
<td>0.1704 0.0482 ( ( p = 0.34 ) )</td>
</tr>
</tbody>
</table>

\( A_{\text{ix}_{\text{bra}}} \), augmentation index on brachial artery; CC, correlation coefficient; CCA, common carotid artery; ICA, internal carotid artery; IMT, intima media thickness; \( PWV_{\text{ao}} \), pulse wave velocity on aorta.
of carotid IMT (0.36). Zhao et al. performed a classical twin study by using 98 middle-aged male twin pairs from the Vietnam Era Twin Registry and demonstrated a significant heritability for carotid IMT (0.59), but because of the twin registry of military veterans, the generalizability to other populations was uncertain. In addition, the analysis included only male and older twin pairs with a relatively small sample size, which had a limited power to detect the influences of common environment on IMT variance. As to the absence of heritability for the IMT of distal right CCA and proximal right ICA, no former studies have had these findings, and further investigation is needed to understand why no genetic influence was found on these two segments. Carotid artery plaques, if present, were registered in the database. Statistically significant differences across countries (between Hungary and Italy, between United States and Italy) observed for some IMT measurements, Aix_{bra} and PWV_{ao} mean values can be mainly attributable to different subject characteristics of involved Italian twins (e.g., older age and higher smoking prevalence). As regards the correlation coefficients between the Aix_{bra}, PWV_{ao} and carotid IMT parameters, there were some slight differences in coefficient values between countries, but currently sample size is too small to draw conclusions.

The genetic influence on IMT (18–38%) and on the arterial stiffness parameters (42–45%) may play a role in the prevention of atherosclerosis. The low, not significant correlation between these variables might indicate that (mainly) increased or abnormal arterial stiffness parameters might mean an initial atherosclerosis that might not be accompanied with increased carotid IMT. This endothelial dysfunction is the early phase of atherosclerosis, which can be detected by arterial stiffness test and treated by appropriate therapy to prevent or postpone serious complications such as stroke and heart attack. Consequently, patients with a positive family medical history of early heart and vascular disease could be checked in the young adulthood. Similar inferences can be drawn from the low but significant heritability of certain IMT parameters, which emphasizes the possible importance of B-mode carotid ultrasound screening in high-risk patients. In addition, it is important to note that the estimated correlation between the carotid IMT and arterial stiffness parameters indicates that increased Aix_{bra} and PWV_{ao} accompanies rarely with increased IMT values. Accordingly, in order to prevent unnecessary and increased number of carotid screening ultrasounds, an appropriate arterial stiffness test should be performed first in high risk patients, possibly by family doctors, radiologists or vascular technicians. In case of increased or abnormal arterial stiffness parameters, carotid ultrasound could be performed because subjects with echogenic plaques have greater PWV_{ao} mean compared with those without plaques.

A worldwide epidemic of cardiovascular and cerebrovascular disease has been anticipated. Estimation of the traditional Framingham risk factors such as age, sex, blood pressure, smoking, and lipid levels is imperfect because they explain only half of the variance in coronary risk. New risk factors have been determined in 2007 Guidelines for the Management of Arterial Hypertension including diabetes mellitus, metabolic syndrome, electrocardiographic (particularly with strain) or echocardiographic (particularly concentric) left ventricular hypertrophy, ultrasound evidence of carotid artery wall thickening or plaque, increased arterial stiffness, moderate increase in serum creatinine, reduced estimated glomerular filtration rate or creatinine clearance, microalbuminuria or proteinuria and established cardiovascular or renal disease.
In addition, approximately one-half of patients who experience a stroke or myocardial infarction have no warning symptoms, and sometimes these are the first and the last symptoms. It would be necessary to have and use noninvasive methods for identifying patients at higher risk by the presence of preclinical atherosclerosis. Several authors have suggested that methods such as carotid IMT, plaque in extracoronary arteries, coronary calcification, wall rigidity in aorta and peripheral arteries, abnormal flow-mediated endothelium-dependent vasodilation, and blood rheology may optimize the management of hypertension.

Noninvasive screening of atherosclerosis should ideally involve methods that are safe, inexpensive, noninvasive, reliable, reproducible, and moreover can be used in a large population. In addition, their results should correlate with the extent of atherosclerotic disease and have high positive and negative predictive value for clinical events. The cost-effectiveness of atherosclerosis scanning has been previously investigated by several studies. found that carotid plaque measurement and progression of plaque may be useful for targeting preventive therapy and may improve cost-effectiveness of secondary preventive treatment. In addition, our results indicate that shared environmental factors do not contribute significantly to carotid IMT in this population as found in other study. Our findings affirmed that unshared environmental factors, maybe active and passive smoking, diet, and physical activity, are largely responsible for the atherosclerosis, which can be preventable in the high-risk patients.

A strength of our study was that all the arterial stiffness tests were performed by the same researchers (ADT and DLT) and device at all places. Furthermore, arterial stiffness tests and carotid IMT measurements were conducted on the same day. Our results were derived from healthy adult twins between age of 18 and 82, and therefore may extend to younger subjects or populations with clinically manifest cardiovascular disease, respectively. In addition, no large (>100 twin pairs) international twin study with a both-genders twin population has investigated the heritability of the carotid IMT parameters. Finally, the multidisciplinary research team analyzed the results and helped in the organization of the study.
There are some limitations to our study. First, the ultrasound measurements were conducted by different devices in the four research places. Second, serum lipid levels were not analyzed in the sample. Third, heritability results are determined by the overall twin sample. Different ethnicity is also a limitation; the overall sample was not homogenous because of the different characteristics of involved Italian twins (e.g., older age and higher smoking prevalence). The recruitment is currently ongoing; therefore, sex and countries differences in the variance components will be analyzed using the complete database.

Conclusions

In summary, the preliminary results of our study indicate that the heritability of arterial stiffness and carotid IMT in an international adult twin sample is moderate. Environmental factors of relevance for these measures appeared not to be shared within family but related to individual experience. The estimated correlation between the parameters is low and not significant. These findings may highlight the genetic and environmental etiology of atherosclerosis and the importance of early atherosclerosis screening, detection and prevention in high-risk patients.

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