Evidence for a Strong Genetic Influence on Carotid Plaque Characteristics

An International Twin Study

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Background and Purpose—Few family studies reported moderate genetic impact on the presence and scores of carotid plaques. However, the heritability of carotid plaque characteristics remains still unclear. Twin studies more reliably estimate the relative contribution of genes to these traits in contrast to family study design.

Methods—One hundred ninety-two monozygotic and 83 dizygotic adult twin pairs (age 49±15 years) from Italy, Hungary, and the United States underwent B-mode and color Doppler ultrasound of bilateral common, internal, and external carotid arteries.

Results—Age-, sex-, and country-adjusted heritability was 78% for the presence of carotid plaque (95% CI, 55%–90%), 74% for plaque echogenicity (hypoechoic, hyperechoic, or mixed; 95% CI, 38%–87%), 69% for plaque size (area in mm^2 in longitudinal plane; < or >50 percentile; 95% CI, 16%–86%), 74% for plaque sidedness (unilateral or bilateral; 95% CI, 25%–90%), 74% for plaque numerosity (95% CI, 26%–86%), 68% (95% CI, 40%–84%), and 66% (95% CI, 32%–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%). Controlling for relevant covariates did not change the results significantly.

Conclusions—The heritability of ultrasound characteristics of carotid plaque is high. Unshared environmental effects account for a modest portion of the variance. Our findings should stimulate the search for genes responsible for these traits. **(*Stroke*. 2012;43:3168-3172.)**

Key Words: carotid atherosclerosis ■ genetics ■ plaque composition ■ twin study

Carotid artery plaques have been associated with multiple complications such as cardiovascular events, retinal or cerebral ischemia,[1] and all-cause mortality.[2,3] Brain or retinal ischemia can be attributable to microembolic dissemination from a complicated plaque or to the hemodynamic consequences of carotid occlusion. Moreover, complex, vulnerable plaques are frequently found in association with hypertension and diabetes, often in subjects without clinically overt cardiovascular disease.[4]

The grade of carotid stenosis related to carotid plaques can be assessed by ultrasound, CT, MRI, or angiography. Carotid artery ultrasound is a well-established method to visualize and

Received June 2, 2012; final revision received August 22, 2012; accepted August 27, 2012.

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The online-only Data Supplement is available with this article at http://stroke.ahajournals.org/lookup/suppl/doi:10.1161/STROKEAHA.112.666016/-/DC1.

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***Stroke* is available at http://stroke.ahajournals.org** **DOI: 10.1161/STROKEAHA.112.666016**

quantify atherosclerotic lesions (composition, vascularization, thickness of cap, plaque motion, and ulceration) and its vulnerability.[3,5,6] The ultrasonographically determined composition of carotid plaques was reported to be a better predictor of adverse events than plaque size.[7] In particular, echo-lucent lipid-rich, histologically "soft" plaques are associated with more complications compared with calcified or mixed plaques.[3,6] Heterogeneous plaques including a hypoechoic component are associated with frequent intraplaque hemorrhage, ulceration, and adverse cardiovascular events.[8–10]

The underlying atherosclerotic phenotypes associated with carotid plaque formation (eg, intima-media thickness, grade of stenosis, arterial stiffness) have moderate heritability according to multiple studies,[11–14] but the genetic effects related directly to carotid plaque characteristics remain unclear. The aim of our study was to assess the heritability of carotid plaque characteristics in a twin population.

Materials and Methods

Subjects and Study Design

Two hundred seventy-five white Italian, Hungarian, and American adult twin pairs (192 monozygotic and 83 dizygotic; age 49±15.5 years, mean±SD) were recruited in this classical twin study. Twin pairs from other ethnicities were not involved in the study. Opposite-sex dizygotic twin pairs were excluded because their inclusion could bias the heritability estimates upward in the presence of sex-specific environmental or X chromosome effects, which could not be modeled because of a limited statistical power. Additional exclusion criteria were pregnancy and individuals with past carotid surgery. Zygosity was assigned according to a 7-part self-reported response.[15,16] The research was conducted in accordance with the Declaration of Helsinki; the local ethical committees approved the study and all subjects gave informed consent before study entry. All subjects were restricted from smoking for 3 hours, from eating for 1 hour, and from drinking alcohol or coffee for 10 hours before measurements. See more details in the Supplemental Data.

Carotid Ultrasonography

The measurement of carotid ultrasound was performed by B-mode and color Doppler ultrasound 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 Mylab40, in the United States: Sonosite Titan). Sonographers were professional internists, neurologists, or radiologists. Bilateral carotid arteries were assessed from the origin of the common carotid artery until the proximal 3 to 4 cm of the internal and external carotid arteries. 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.[17] The investigators assessed bilaterally the presence, sidedness, number of carotid plaques, and their size (mm^2) on common carotid artery, proximal internal carotid artery, and external carotid artery. See more details in the Supplemental Data.

Statistical Analysis

Descriptive analysis (mean±SD for continuous variables, percentage for categorical variables) for age, zygosity, smoking history, body mass index, and the investigated carotid plaque characteristics was conducted using SPSS (SPSS 17.0 for Windows; SPSS, Chicago, IL). Differences among sex, zygosity, and countries were calculated using independent-sample *t* tests. *P* value <0.05 was considered significant. Due to the zero-inflated distribution of the plaque area values, 3 categories were created and analyzed as ordinal: no plaque and < or >50 percentile. To investigate whether the anthropometric or cardiovascular risk factors have a substantial influence on the carotid plaque parameters beyond age, sex, and country, bivariate probit regression analyses were performed. The corresponding covariates have been added to the list of possibly relevant covariates in the heritability models. A descriptive estimate of the genetic influence was calculated using the within-pair correlation in monozygotic and dizygotic twin pairs with the corresponding bootstrapped 95% CIs.[18] Structural equation modeling was used to estimate heritability using the Mplus Version 6.1 (Muthén and Muthén) weighted least squares estimation due to the categorical nature of variables of interest.[19] Empirical CIs were calculated with a Bollen-Stine bootstrap procedure.[20] For each phenotype 2 ACE models were estimated. Model 1 follows convention and corrects for the twins' age, sex, and country. Model 2, in addition to age, sex, and country, also corrects for all risk factors with a significant relationship. These results tell us the impact of genes and of the environment after the influence of known risk factors is controlled for. See more details in the Supplemental Data.

Results

Clinical Characteristics

Supplemental Table 1 presents clinical characteristics of the study population by zygosity, sex, and country. Seventy percent of the twins were monozygotic. Dizygotic twins were older and had higher active smoking rate ($P<0.05$). Seventy-one percent of the subjects were female. Carotid plaque prevalence and size and body mass index were higher in males compared with females (all $P<0.05$). Significant differences were detected for age of participants, plaque area, and smoking rate across countries ($P<0.05$). These differences were taken into account in cotwin correlation estimation and quantitative genetic models by regressing out age, sex, country (in Model 1), and significant covariates (in Model 2).

The age, sex, country, zygosity, and family corrected probit regression coefficients of the investigated carotid plaque characteristics with anthropometric measurements and cardiovascular risk factors are shown in Supplemental Table 2. Some carotid plaque characteristics, smoking, and age showed a positive relationship. On the other hand, no association (2 exceptions: presence of carotid plaque and diabetes; plaque sidedness and hyperlipidemia) was observed between carotid plaque characteristics and sex, country, body mass index, central blood pressure, diabetes, and hyperlipidemia (Supplemental Table 2).

Heritability Analysis

Cotwin correlations as well as genetic and environmental proportions of variance, and their 95% CIs, are shown in the Table. In Model 1 (with only age, sex, and country adjusted for), additive genetic factors appear to contribute highly to all investigated carotid plaque phenotypes: 78.0% for the presence of carotid plaque (95% CI, 54.5%–90.3%), 74.3% for plaque echogenicity (95% CI, 38.3%–87.3%), 69.8% for plaque area (95% CI, 15.5%–85.9%), 74.0% for plaque sidedness (95% CI, 25.4%–89.8%), 73.5% for plaque numerosity (95% CI, 26.0%–86.2%), and 67.5% (95% CI, 40.4%–84.0%) and 66.0% (95% CI, 32.3%–90.2%) for the presence of plaques in carotid bulbs and proximal internal carotid arteries. No role of shared environmental factors was found. Unshared environmental effects accounted for the additional minor part of the variance (22.0%–34.0%).

Table. Cotwin Correlations and Genetic and Environmental Variance Components as Estimated Under the Univariate ACE Models in Percentage

Measure	Models	Twin Correlations		Variance Components		
		*r*MZ	*r*DZ	A	C	E
Presence of carotid plaque	Model 1 ACE	0.788 (0.560–0.914)	0.205 (–0.610 to 0.719)	78.0% (54.5–90.3)	0.0% (0.0–0.0)	22.0% (9.6–44.7)
	Model 2 ACE	0.802 (0.537–0.928)	0.208 (–0.771 to 1.000)	79.2% (54.2–92.0)	0.0% (0.0–0.0)	20.8% (8.0–44.9)
Plaque composition	Model 1 ACE	0.754 (0.553–0.876)	0.121 (–0.426 to 0.519)	74.3% (38.3–87.3)	0.0% (0.0–0.0)	25.7% (13.3–45.0)
	Model 2 ACE	0.715 (0.522–0.855)	0.114 (–0.450 to 0.615)	70.4% (36.8–84.3)	0.0% (0.0–75.9)	29.6% (16.1–48.5)
Plaque area*	Model 1 ACE†	0.698 (0.448–0.837)	0.258 (–0.499 to 0.798)	69.1% (15.5–85.9)	0.0% (0.0–39.3)	30.9% (17.3–52.7)
Plaque sidedness	Model 1 ACE	0.741 (0.531–0.852)	0.346 (–0.218 to 0.738)	74.0% (25.4–89.8)	0.0% (0.0–68.9)	26.0% (14.6–45.5)
	Model 2 ACE	0.700 (0.503–0.834)	0.402 (–0.224 to 0.990)	59.7% (0.0–81.0)	10.3% (0.0–77.9)	30.0% (16.0–48.2)
Plaque quantity	Model 1 ACE	0.745 (0.564–0.835)	0.267 (–0.421 to 0.652)	73.5% (26.0–86.2)	0.0% (0.0–48.8)	26.5% (17.2–42.8)
	Model 2 ACE	0.726 (0.541–0.840)	0.264 (–0.467 to 0.661)	71.4% (34.9–91.1)	0.0% (0.0–73.3)	28.6% (16.7–43.7)
Presence of plaque in carotid bulbs	Model 1 ACE	0.708 (0.459–0.877)	–0.259 (–1.000 to 0.281)	67.5% (40.4–84.0)	0.0% (0.0–0.0)	32.5% (16.1–56.8)
	Model 2 ACE	0.740 (0.416–0.874)	–0.176 (–1.000 to 0.442)	71.6% (9.4–86.6)	0.0% (0.0–0.0)	28.4% (14.0–54.1)
Presence of plaque in internal carotid arteries	Model 1 ACE	0.667 (0.337–0.841)	0.228 (–0.998 to 1.000)	66.0% (32.3–90.2)	0.0% (0.0–0.0)	34.0% (13.7–57.9)
	Model 2 ACE	0.625 (0.316–0.804)	0.193 (–1.019 to 0.760)	61.7% (32.7–83.9)	0.0% (0.0–53.1)	38.3% (16.1–63.0)

Model 1 results are age-, sex-, and country-adjusted. Model 2 results, in addition, adjust for significant covariates. Numbers in parentheses are 95% CIs.
*r*MZ indicates monozygotic correlation; *r*DZ, dizygotic correlation; A, heritability; C, shared environmental variance component; E, unique environmental variance component.

*Based on 240 twin pairs only (American subjects not included in the analysis).

†No Model 2 because none of the covariates was significant.

In multivariable-adjusted heritability estimates (Model 2 ACE), the magnitude of the genetic and environmental effects has not changed significantly.

Discussion

To the best of our knowledge, this is the first study that investigated heritability of carotid plaque characteristics (presence, numerosity, sidedness echogenicity, area) in an international twin population. We have shown that heritability is high and the influence of unique environmental factors is moderate.

Only few studies have investigated, indeed partially, the genetic determinants of carotid plaque characteristics.[12,21,22] Moskau et al[12] reported no genetic base for the carotid plaque score but demonstrated a moderate heritability of maximal carotid stenosis using a family study design; however, the sample size was small. A moderate (23%–28%) heritability of the presence of carotid plaques was reported in the San Antonio Family Heart Study.[21] Their analysis was limited to the largest plaque identified in either the carotid bulb or internal carotid artery only; this might explain the lower heritability compared with our findings. Twenty-eight percent heritability of the carotid plaque score was found in the Erasmus Rucphen Family study by measuring the common carotid artery, carotid bifurcation, and internal carotid artery.[22]

It must be taken into consideration that the family design is useful in the determination of intergeneration resemblance or difference, but in contrast to the twin study design, it does not tangibly express outside factors such as family environment and culture.[23] Family studies, especially the ones limited to 2 generations, make more assumptions than the classical twin design concerning the sources of within-family transmission of a trait. Two-generation studies cannot reliably distinguish heritability from vertical cultural transmission and 3-generation designs still make several assumptions about the family environment. Our twin study reports higher genetic heritability than the cited family studies on additional key carotid plaque phenotypes. Our findings indicate that common environmental factors are not responsible for the total variance of these phenotypes, whereas unshared environmental factors affecting individual twins separately have a moderate impact. One possible explanation for the sizably higher heritability of the study at hand is better plaque measurements thanks to more advanced ultrasound devices.

A genomewide linkage analysis obtained a bit higher heritability estimates for plaque presence (50%),[24] yet these kind of studies usually underreport heritability.[25,26] Noteworthy, their heritability CIs overlapped with ours. The same study identified loci on chromosomes 11p15, 14q32, and 15q23, which might influence heritability and stated that the *SOX6* gene within the bone morphogenic protein pathway could be a candidate for carotid plaque formation.[24] The heterogeneity of percentage heritability reported in the various studies might derive from dissimilar study designs, different study populations, adjustments for various covariates, and population-specific environmental contributions to the phenotypic variances. Key findings of the family, twin, and genomewide linkage analysis studies investigating carotid plaque heritability are summarized in Supplemental Table 3.

In our study, heritability of carotid plaque characteristics was much higher than previously reported in family studies, ranging between 66% and 78%.[12,21,22] We hypothesized that the genetic influence on these traits might be similar to other functional and structural parameters of the arterial wall such as arterial stiffness (characterized by carotid pulse wave velocity or augmentation index), central blood pressure, or carotid intima-media thickness[11,13,14] or to some cardiometabolic risk factors, which are known to be moderately heritable.[27–29] In a previous work, we investigated the heritability of vascular parameters on a smaller twin cohort and found that low to moderate genetic variance is responsible for the determination of these traits with heritabilities ranging between 0% and 38%

for segment-specific carotid intima-media thickness, 45% for brachial augmentation index, and 42% for aortic pulse wave velocity assessed by oscillometry (TensioMed Arteriograph).[13] In contrast to those heritability estimates, in the present study we found an unexpectedly high genetic influence on the investigated carotid plaque traits. Of note, we found no influence of additional cardiovascular and anthropometric covariates on the heritability analyses (Model 2 ACE). The Healthy Twin Study provided evidence for segment-specific heritability of carotid intima-media thickness (heritability of intima-media thickness was 48% for common, 38% for carotid bifurcation, and 45% for internal carotid artery, respectively) and a shared genetic variation was reported on the 3 carotid segments.[30] Therefore, we also investigated whether the presence of carotid plaques are segment-specific in our sample. Because the number of plaques on the proximal common and external carotid arteries was too low, only the heritability of the carotid plaques in the bulbs and proximal internal carotid arteries was calculated, which indicated also higher heritability estimates (67.5% and 66.0%, respectively). These findings should stimulate the search for novel genes responsible for these promising candidate phenotypes in atherosclerosis.

Only 1 twin study has dealt with plaques so far, namely calcified aortic plaques assessed by CT, and reported a similarly high heritability (61%).[30] In addition, Cecelja and coworkers[31] suggested that the association between aortic wall calcification and increased arterial stiffness is explained by a common genetic background.

If our finding of a high genetic determinacy of carotid plaque characteristics is confirmed by future studies, carotid ultrasound might represent a very useful screening method in individuals with a family history of early cardio- and cerebrovascular events. A worldwide epidemic of cerebrovascular disease has been anticipated[32] and ultrasound evidence of carotid artery wall thickening or plaque is considered a key measure of target organ damage in hypertensive subjects.[33] In addition, arterial stiffness assessment might be helpful in identifying high-risk individuals, because atherosclerotic plaque formation can be genetically associated with arterial stiffening.[31]

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[34,35] still remains important, because in societies where everyone is exposed to cardiovascular risk factors, genetics will become relatively more important in determining who will be affected by carotid atherosclerosis (ie, high heritability), yet management of cardiovascular risk factors remains the cornerstone to eradicating this disease, because these risk factors might be necessary for the disease to appear. Accordingly, these environmental factors might contribute to the atherosclerotic plaque composition and vulnerability modestly. However, environmental factors might be more relevant in genetically susceptible individuals, and therefore gene×environment interactions—not modeled here for reasons of power—should be investigated in future studies.

Potential limitations of our study should be considered: (1) carotid plaque size was assessed only in 2 dimensions; (2) the proportion of dizygotic twins was relatively small compared with other twin studies, which may lead to biased estimates in quantitative genetic analysis; (3) carotid ultrasound reading was not blinded to monozygotic/dizygotic status, which may increase our heritability estimates; (4) the study was carried out in cohorts of different ethnicity. However, no evidence of heterogeneity between countries was detected, and all the analyses were adjusted for country; (5) the unique environmental factor (E) category can be biased upward by random measurement error; and (6) the sample size did not allow us to test for sex differences in genetic and environmental variance components or to explore gene×environment interactive effects.

Conclusion

Our study showed for the first time in adult twins that the heritability of the key carotid plaque characteristics is high. Unshared environmental effects account for a modest portion of the variance. These findings, if validated by other laboratories, should stimulate the search for genes responsible for these traits.

Acknowledgments

We acknowledge the support of the Twins Days Festival committee and Stephen F. Luczek, MD, for the American part of the study.

Sources of Funding

Medexpert Ltd has provided financial support for the development and maintenance of this study (financial support for the American and Perugian measurements). The Italian part of the research was supported by the Balassi Institute–Hungarian Scholarship Board Office, Foreign Affairs of Republic of Italy.

Disclosures

Dr Tarnoki received travel reimbursement from Medexpert Ltd (American and Perugian measurements). Dr Garami is in the advisory board of Edwards (TAVI) and W.L. Gore (CAS-TCD).

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