Genetic Influences on Peripheral Arterial Disease in a Twin Population

Carl Magnus Wahlgren, Patrik K.E. Magnusson

Objective—To analyze the contribution of genetic and environmental factors to the development of peripheral arterial disease (PAD) in a large population-based sample of twins.

Methods and Results—The understanding of genetics in PAD is still limited. From the Swedish Twin Registry and the national patient discharge registry, 1464 twins with PAD were identified, including 33 monozygotic (MZ) and 42 dizygotic (DZ) concordant pairs and 298 MZ and 1008 DZ discordant pairs. Traditional cardiovascular risk factors were significantly more prevalent in twins with PAD than in those without PAD. Concordances and correlations were higher in MZ compared with DZ twins, indicating genetic influences in PAD. The risk of PAD for persons whose twin had PAD compared with persons whose twin did not have PAD, estimated as an odds ratio, was 17.7 (95% CI, 11.7 to 26.6) for MZ twins and 5.7 (95% CI, 4.1 to 7.9) for DZ twins. In the structural equation models, Mx analyses, genetic effects accounted for 58% (95% CI, 50% to 64%) and nonshared environmental effects for 42% (95% CI, 36% to 50%) of the phenotypic variance among twins.

Conclusion—Heritability is an important component, along with unique environmental factors, for the development of PAD. The proportion of the overall PAD heritability attributed to the heritability of cardiovascular risk factors needs to be resolved. Traditional risk factors could explain a major proportion of PAD heritability. A better understanding of the genetics in PAD could identify individuals at increased risk who may benefit from targeted therapies. (Arterioscler Thromb Vasc Biol. 2011;31:00-00.)

Key Words: epidemiology ■ peripheral arterial disease ■ environmental factors ■ heritability ■ twins

The prevalence of lower-extremity peripheral arterial disease (PAD), based on objective testing, has been estimated to be 3% to 10%, increasing to 15% to 20% in persons older than 70 years.1,2 In its milder forms, PAD may be asymptomatic (eg, a reduced ankle-brachial index [ABI]) or limited to intermittent claudication. However, when ischemia in those with PAD is chronic or critical, the risk of gangrene, amputation, and premature death is increased. Patients with PAD have multiple atherosclerosis risk factors and extensive atherosclerotic disease, placing them at markedly increased risk for cardiovascular events.3 In particular, hypertension, cigarette smoking, and diabetes mellitus are powerful risk factors for the development of PAD.4 All patients with PAD should receive risk factor modification, such as smoking cessation, blood pressure control, and lowering of cholesterol; and appropriate pharmacological management, including antplatelet therapy.5

PAD is a common and complex trait, of which development is probably modified to some degree by uncharacterized genetic determinants that are independent of traditional risk factors of atherosclerosis.6 Family studies of PAD are necessary to better estimate the relative importance of genetic and nongenetic determinants of disease. In recent years, genetics has profoundly changed our understanding of several cardiovascular diseases.7,8 However, our understanding of the genetic basis of PAD is limited. The aim of this study was to analyze the contribution of genetic and environmental factors to the development of PAD in a large population-based sample of twins.

Methods

Data Sources and Study Design

The Swedish Twin Registry (STR) was established in the late 1950s and contains data on almost all twins born in Sweden from 1886 to 1990.9 The registry has been expanded and updated on several occasions. The classical twin method is based on the fact that monozygotic (MZ) twins share 100% of their genome, whereas dizygotic (DZ) twins share, on average, 50% of their segregating genes.10 Twin similarity in the STR was determined by asking twins whether they and their twin partner were similar as “2 peas in the pod” during childhood, more than siblings in general. If both twins answered that they were similar as 2 peas in the pod, they were classified as MZ twins. Twin pairs for which both twins answered that they were not more similar than siblings in general were classified as DZ twins. Those who answered differently were classified as not determined zygosity.
ity (response to baseline questionnaires and interviews) have been validated by genetic testing in the registry several times, with an accuracy of 95% to 99%.7 In the Screening Across the Lifespan Twin study, initiated in 1998 with the purpose of screening all twins born before 1959 for most common complex diseases, whole blood samples for DNA extraction and serum samples for clinical chemistries were obtained. For 2836 (6%) of the twin pairs included in this study, the zygosity has been determined by genotyping 47 SNP markers.11 Analyses of the relative importance of genes and environment for a phenotype can be performed with traditional measurement methods that are well developed for twin studies. The National Board of Health and Welfare, and reporting is mandatory. PAD, referred to as lower-extremity atherosclerotic disease, was coded according to the International Classification of Diseases (ICD)-8 between 1964 and 2006) (NPR, The Centre for Epidemiology, The National Board of Health and Welfare, Stockholm, Sweden; http://www.socialstyrelsen.se). In 2003, the main diagnosis was missing in 0.9% of the hospital admissions reported. The diagnoses coded according to the International Classification of Diseases (ICD)-8 between 1969 and 1986, the ICD-9 between 1987 and 1996, and the ICD-10 between 1997 and the present were used. The NPR is continuously validated by the National Board of Health and Welfare, and reporting is mandatory. PAD, referred to as lower-extremity atherosclerotic disease, was coded as 440.20 in ICD-8, 440C in ICD-9, and 170.2 in ICD-10.

The 2 registries (STR and NPR) were cross-linked using the national 10-digit social security number, a unique number assigned to every Swedish citizen. The STR database is regularly updated with information about inpatient diagnoses and hospital discharges. For risk factors, data were also collected from several questionnaires during the 1960s and 1970s and the Screening Across the Lifespan Twin study telephone interviews covering twins born from 1886 to 1958.12 The study was approved by the local research ethics committee and the STR Board.

Prevalence
The prevalence of PAD was calculated as a percentage of individual twins diagnosed in the entire twin cohort and the MZ and DZ groups of twins separately.

Probандwise Concordance
Twin similarity was calculated as probandwise concordance rates for both zygosity groups. Concordance rates represent the probability of developing PAD for an individual with an affected twin. Concordant pairs are in pairs in which both twins experienced the event being studied. Discordant pairs are in pairs in which 1 twin experienced the event being studied.

Concordance rate
\[
\frac{2 \times \text{concordant affected pairs}}{2 \times \text{concordant affected pairs} + \text{discordant pairs}}
\]
When MZ concordance rates are greater than DZ concordance rates, genetic influences are indicated. If the concordance is similar for both types of twins and higher than expected by chance, then shared environmental effects are indicated.

Pairwise concordance is defined as follows: \(C(C+D)\), where C is the number of concordant pairs and D is the number of discordant pairs.

Odds Ratio
The relative risk of PAD for persons whose twin had PAD compared with persons whose twin did not have PAD was estimated as an odds ratio and was calculated as follows:

\[
\text{Odds Ratio} = \frac{(a \times d)}{(b \times c)}
\]
where \(a\) is the number of concordant pairs, \(b\) and \(c\) are each half the number of discordant pairs, and \(d\) is the number of discordant pairs without PAD.

Intraclass Correlation and Heritability
Tetrachoric correlations were calculated assuming an underlying binormal distribution of liability, with multiple factors contributing additively and a threshold value that discriminates between presence and absence of PAD. Differences in correlations between various groups provide information about the presence of genetic effects. Higher correlations of liability in MZ than in DZ twins suggest that genetic factors influence disease development.

Structural equation models can estimate the proportion of variation of shared genetic and environmental influences in a phenotype.12 According to standard biometric practice, no gene–gene interaction, no gene–environment interaction or correlation, random mating, and the assumption that twins are representative of the general population were all assumed.12 The total variance in the trait can be partitioned into additive genetic variance \((a^2)\), shared (familial) environmental variance \((c^2)\), and nonshared (unique) environmental variance \((e^2)\). The method for selecting the best model followed standard procedures by using Mx structural equation analyses.13 The Akaike information criterion was used to compare the goodness of fit between nonnested models (eg, ACE and ADE). The principle of parsimony was implemented to determine which nested model was to be preferred (eg, ACE or AE) when the \(x^2\) test was not significant \((P<0.05)\). To estimate the parameters of interest, the equation for 1 of the twins can be written as follows:

\[
V_p = a^2 + c^2 + e^2 = 1
\]
\(V_p\) is the total phenotypic variance of the population, representing the sum of the individual components \(a^2\), \(c^2\), and \(e^2\). Heritability, the relative importance of genetic influences for variation in a trait, is defined as additive genetic variance \((a^2)\) divided by total phenotypic variance.

We could estimate the contribution of genetic (A) and shared (C) and nonshared (E) environmental effects by using the full biometric model (ACE) and its submodel (AE). Because the effects of genetic dominance and shared environment are confounded in the classical study of twins reared together, it is not possible to estimate those parameters simultaneously in a single model.14 Shared environmental factors contribute to similarity in pairs of twins (eg, same intrauterine environment, passive smoking in childhood family, or similar dietary habits). Nonshared environmental factors (eg, sporadic mutations, infections, or occupational exposure) will lead to a decrease in twin similarity.

Statistical Software
Structural equation modeling was performed with Mx analyses, a software package specifically designed for analysis of genetically informative data. \(P<0.05\) was considered statistically significant.

Results
In the STR, 95 628 twins (47 529 complete pairs: 12 216 MZ pairs and 35 313 DZ pairs) with known zygosity were born between 1886 and 1970. There were 1464 twins with PAD (49.7% were men; mean age, 73 years; age range, 35 to 104 years). The prevalence for the entire twin cohort was 1.53%; MZ twins, 1.49%; and DZ twins, 1.55%. Among concordant pairs, there were 33 MZ (52% female) pairs and 42 DZ (36% male, 33% female, and 31% opposite-sex) pairs with PAD. The corresponding numbers for discordant pairs were 298 MZ (56% female) pairs and 1008 DZ (28% male, 29% female, and 43% opposite-sex) pairs with PAD. There was a small difference between MZ and DZ twins regarding age of PAD diagnosis (mean age
Table 1. Risk Factors for Twins With or Without PAD*

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>PAD (n=727)</th>
<th>No PAD (n=42 322)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes mellitus</td>
<td>181 (24.9)</td>
<td>2073 (4.9)</td>
<td>&lt;.00001</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>135 (18.6)</td>
<td>1603 (3.8)</td>
<td>&lt;.00001</td>
</tr>
<tr>
<td>Heart failure</td>
<td>92 (12.7)</td>
<td>1327 (3.1)</td>
<td>&lt;.00001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>339 (46.6)</td>
<td>9925 (23.5)</td>
<td>&lt;.00001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>171 (23.5)</td>
<td>5042 (11.9)</td>
<td>&lt;.00001</td>
</tr>
<tr>
<td>Smoker (previous or current)</td>
<td>569/936 (60.8)</td>
<td>22 953/47 661 (48.2)</td>
<td>&lt;.00001</td>
</tr>
</tbody>
</table>

*Data are from questionnaires covering twins born between 1886 and 1958. †Data are given as number (percentage) of each group, except for smoker.

Table 2. Twin Similarity and Correlations of Liability for PAD

<table>
<thead>
<tr>
<th>Zygosity</th>
<th>Concordant Pairs With No PAD</th>
<th>Concordant Pairs With PAD</th>
<th>Discordant Pairs With PAD</th>
<th>Probandswise Concordance</th>
<th>Tetrachoric Correlation (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monozygotic</td>
<td>11 885</td>
<td>33</td>
<td>298</td>
<td>0.181</td>
<td>0.55 (0.43–0.64)</td>
</tr>
<tr>
<td>Dizygotic</td>
<td>34 263</td>
<td>42</td>
<td>1008</td>
<td>0.077</td>
<td>0.32 (0.25–0.39)</td>
</tr>
</tbody>
</table>

Table 3. Estimation of Genetic and Environmental Effects on PAD*

<table>
<thead>
<tr>
<th>Model</th>
<th>A</th>
<th>C</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE</td>
<td>48 (26–64)</td>
<td>7.7 (0–24)</td>
<td>44 (36–53)</td>
</tr>
<tr>
<td>AE</td>
<td>58 (50–64)</td>
<td>NA</td>
<td>42 (36–50)</td>
</tr>
</tbody>
</table>

*Data are given as (95% CI) percentage.

Discussion

PAD is a common manifestation of systemic atherosclerosis associated with a high risk of morbidity and mortality from cardiovascular events. Despite this, PAD is often undiagnosed and, therefore, undertreated. PAD is common in Sweden, and almost a fifth of all elderly individuals have some stage of this disease. Women have a higher prevalence than men when PAD is diagnosed using the ABI.

Wahlgren and Magnusson Genetics of PAD

The twin model provides a powerful means of examining the total genetic contribution to a given disease, especially a complex trait such as PAD. In this largest PAD population-based twin study to date, we provide epidemiological evidence that heritability contributes to PAD development. Concordances and correlations were significantly higher in MZ compared with DZ twins, indicating genetic effects. There was an 18% probability that the MZ twin of a person with PAD would have the disease. The twin of an MZ twin with PAD had a risk of PAD that was almost 18 times that of the MZ twin of a person without PAD. A heritability of 58% of the total trait variance was estimated. The remaining variance was explained by nonshared environmental factors, with only a small role for shared environmental influences.

The traditional cardiovascular risk factors were significantly more prevalent in twins with PAD than in those without PAD. Only a subset of this study cohort was represented in the questionnaires including the risk factors. However, we believe the result reflects the expected distribution of risk factors in this PAD twin cohort. The proportion of overall PAD heritability attributed to the heritability of traditional risk factors is of great importance. It is possible, and maybe even likely, that genetic regulation of these risk factors explains a major proportion of the PAD heritability that may make identification of specific genetic determinants for PAD challenging. Even if causative mechanisms are not possible to disentangle from these types of data, it certainly represented in the questionnaires including the risk factors.

Unfortunately, given the long time between collection of risk factor data and outcome and the different nature data sources, we have not found a model well suited to investigate this question, which is a limitation of this article.

There are 3 previous family studies in the literature reporting estimates of PAD heritability. The ABI has been used as a surrogate for the presence and severity of PAD in these studies. Carmelli et al investigated the contribution of genetic and environmental influences to normative ABI values in a twin population that included 94 MZ and 90 DZ pairs. Structural equation modeling indicated that 48% of
the observed variability in ABI values could be attributed to additive genetic effects. In contrast, concordance rates for low ABI values (≤0.9) for both MZ and DZ twins (concordant pairs: MZ, n=3; and DZ, n=2; and discordant pairs: MZ, n=12; and DZ, n=9) were significantly greater than would be expected by chance alone; however, within-pair MZ similarity was not significantly greater than DZ similarity. The 2 other family studies18,19 showed only modest heritability. Most patients in the previously mentioned studies had only slightly abnormal ABI values. An index of approximately 0.9 has weak correlation to the degree of lower-extremity atherosclerosis.20 Therefore, these studies may better reflect the degree of genetic influence of ABI in the reference range, which may differ significantly from the degree of genetic influence on PAD.6

Unlike family studies or the study of sibling pairs, potential confounders (eg, the variability of disease prevalence with age) are removed in twin studies.21 The twin model approach relies on previously described important assumptions (ie, random mating, no interaction between genes and environment, and equivalent environments for MZ and DZ twins) that potentially could overestimate or underestimate the genetic and environmental components.22 Our estimates are population specific. In other regions, the proportions of the type of effects could differ because of different environmental factors. For PAD with several genetic and environmental factors, the liability model assumes that the disease will occur when there are enough contributory factors to push the individual’s liability higher than the threshold.21

The degree of limb ischemia in this patient cohort is unknown. The PAD diagnosis in this twin population could be underestimated. The Swedish Inpatient Register provided full coverage of hospital discharges after 1987. Therefore, there is a possibility that some twins who were diagnosed as having PAD before 1987 were not recorded as such in the register.

Genetic studies provide new insights into the pathogenesis of PAD. A genetic marker for PAD may help identify people at risk for PAD, improve knowledge about atherosclerosis in the lower extremities, and ultimately lead to new and better therapies for PAD.23 The development of PAD is probably determined by several genes that act collectively, and allelic variants in different genes may have either additive or contrasting effects.24 No definitive genetic markers have been identified for PAD. Genetic factors may have an independent effect and may exacerbate the effect of smoking on the development of atherosclerosis in the peripheral vasculature.25 A genomewide analysis of smokers identified a genetic variant on chromosome 15q24 in the nicotine acetylcholine receptor gene cluster that was associated significantly with both quantity of cigarette smoking and presence of PAD.26 New collaborative candidate gene studies and genomewide association studies are necessary to further elucidate the genetics of PAD.27

In conclusion, heritability is an important component, along with unique environmental factors, for the development of PAD. This study has analyzed the contribution of hereditary and environmental factors for PAD development. It emphasizes the importance of evaluating family history in patients with PAD. Screening of unaffected siblings (ie, identifying individuals at increased cardiovascular risk who may benefit from targeted medical therapies) may be considered but needs to be evaluated in clinical trials. Several questions about the genetics of PAD remain unanswered, and more studies are necessary to better estimate the relative importance of genetic and nongenetic determinants of the disease.

Disclosures
None.

References

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