Polymorphism in the Promoter Region of the Insulin-like Growth Factor I Gene Is Related to Carotid Intima-Media Thickness and Aortic Pulse Wave Velocity in Subjects With Hypertension

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Background and Purpose—Low circulating levels of insulin-like growth factor I (IGF-I) have been associated with an increased risk for atherosclerosis. Absence of the 192-bp (wild-type) allele in the promoter region of the IGF-I gene has been associated with low circulating IGF-I levels. We examined the role of this polymorphism in relation to blood pressure and 2 early markers of atherosclerosis: carotid intima-media thickness (IMT) and aortic pulse wave velocity (PWV).

Methods—A total of 5132 subjects of the Rotterdam Study, aged 55 to 75 years, were included in this study. In 3769 subjects who did not use blood pressure–lowering medication, the association between the IGF-I polymorphism and blood pressure was examined. In the total population, and in 3484 normotensive subjects, 1648 hypertensive and 462 untreated hypertensive subjects, the association between this polymorphism and IMT and PWV was examined.

Results—Mean systolic and diastolic blood pressure did not differ between genotypes. In hypertensive subjects IMT was significantly increased in noncarriers of the 192-bp allele (0.83 mm) compared with heterozygous or homozygous carriers (0.80 mm) ($P=0.04$). PWV was also significantly higher in hypertensive subjects who were noncarriers of the 192-bp allele (14.3 m/s) compared with heterozygous (14.1 m/s) or homozygous carriers (13.7 m/s) ($P=0.02$). Findings were more pronounced in hypertensive subjects without medication use. In normotensive subjects, no association between this polymorphism, IMT, and PWV was observed.

Conclusions—Our study suggests that hypertensive subjects who have low IGF-I levels because of a genetic polymorphism in the IGF-I gene are at increased risk of developing atherosclerosis. (Stroke. 2003;34:1623–1627.)

Key Words: atherosclerosis • cardiovascular diseases • genetics • growth factors • hypertension • risk factors

Insulin-like growth factor I (IGF-I) may play an important role in the development of cardiovascular disease. Its contribution to the development of atherosclerosis is a topic of increasing interest in both human and animal studies. Low circulating IGF-I levels have been associated with the early development of cardiovascular disease. Because of its growth-mediating and vasodilating properties, IGF-I is assumed to be an important mediator in the pathophysiological response to increased blood pressure in the vessel wall. Animal studies have shown that an increase in hemodynamic load is accompanied by increased IGF-I expression in both cardiac and vascular tissues. We have recently demonstrated that a polymorphism in the promoter region of the IGF-I gene is associated with serum IGF-I levels. In our studies absence of the 192-bp (wild-type) allele was associated with 20% lower circulating IGF-I serum levels at middle age, lower body height, and a reduction in birth weight. This polymorphism can be used to study subjects with a genetic predisposition toward chronic low exposure of IGF-I in all tissues of the body, including those of cardiovascular origin.

In this population-based study we examined the effect of this polymorphism on blood pressure and the development of atherosclerosis. We used 2 early markers of atherosclerosis in this study: intima-media thickness (IMT) of the carotid arteries and aortic pulse wave velocity (PWV).

Subjects and Methods

Study Population
The study was performed within the Rotterdam Study, a single-center prospective follow-up study, in which all residents aged ≥55
years of the Rotterdam suburb Ommoord were invited to take part. The baseline examination of the Rotterdam Study was conducted between 1990 and 1993. The study was approved by the Medical Ethics Committee of Erasmus Medical Center Rotterdam. Written informed consent was obtained from all participants. The design of the study has been described previously.\textsuperscript{14}

We examined 7983 participants (response 78%). Because no DNA was available for 948 subjects and in 23 subjects genotyping failed, 7012 subjects were successfully genotyped for the IGF-I gene. Since serum IGF-I was assessed in a subgroup of 150 subjects, consisting of 50 subjects randomly drawn from each genotype group. Since serum IGF-I levels show an age-dependent decline and are related to the development of cardiovascular disease, we excluded all subjects aged >75 years to avoid any bias in our results because of selective mortality. Therefore, only subjects aged 55 to 75 years at baseline examination and successfully genotyped were included in this study (n=5132).

In a subgroup of 3769 subjects who did not use blood pressure-lowering medication, the association between the IGF-I polymorphism and systolic and diastolic blood pressure was examined. In the total study population (n=5132), the IGF-I polymorphism was examined in relation to IMT and PWV. This relation was also examined separately in a subgroup of 1648 hypertensive subjects and in a subgroup of 3484 normotensive subjects. Since lowering of blood pressure is known to influence the development of atherosclerosis, the relation between the IGF-I polymorphism, IMT, and PWV was also assessed in 462 hypertensive subjects who did not use any blood pressure-lowering medication.

**Measurements**

At the baseline examination, information concerning medical history, medication use, and smoking behavior was obtained with a computerized questionnaire.\textsuperscript{14} Height and weight were measured, and body mass index (BMI) (in kg/m\textsuperscript{2}) was calculated. Blood pressure was measured with the subject in the sitting position at the right upper arm with the use of a random zero sphygmomanometer. The average of 2 measurements was used for analysis. Hypertension was defined as a diastolic blood pressure of \(\geq100\) mm Hg and/or a systolic blood pressure of \(\geq160\) mm Hg.\textsuperscript{15} Total serum cholesterol and HDL cholesterol were determined with an automated enzymatic procedure.\textsuperscript{16} Total IGF-I levels were determined in nonfasting serum by a commercially available radioimmunoassay (Medgenix Diagnostics, with an intra-assay and interassay variation of 6.1\% and 9.9\%, respectively). IMT of the left and right carotid artery was assessed by ultrasound.\textsuperscript{17,18} The beginning of the dilatation of the distal common carotid artery served as a reference point for the start of the measurement, and IMT was measured over an average distance of \(10\) mm. The lumen-intima interface and the media-adventitia interface of the near and far walls of the distal common carotid artery were measured offline. In each subject, mean IMT of far and near walls [(left+right)/2] was taken as measure for wall thickness of the distal common carotid artery. The intra- and interclass correlation coefficient for assessment of common carotid IMT was 0.74.\textsuperscript{19} Carotid-femoral PWV was assessed with the use of an automatic device (Complior, Colson) that recorded the time delay between the rapid upstroke of the feet of simultaneously recorded pulse waves in the carotid artery and femoral artery.\textsuperscript{20} The distance traveled by the pulse between the carotid and the femoral artery was measured over the surface of the body with a tape measure. PWV was calculated as the ratio between the distance traveled by the pulse wave and the foot-to-foot delay and was expressed in meters per second. We used the average of at least 10 successive measurements, to cover a complete respiratory cycle, in the analysis. The intra- and interclass correlation coefficient for carotid-femoral PWV was 0.80.\textsuperscript{19} The IGF-I gene promoter polymorphism was genotyped as described elsewhere.\textsuperscript{12} On the basis of our previous studies, 3 genotype groups were distinguished: homozygous carriers of the 192-bp allele, heterozygous carriers, and noncarriers of this allele.

**Data Analysis**

Hardy-Weinberg equilibrium of the IGF-I promoter polymorphism genotypes was tested with the use of the GENEPOP package (M. Raymond and F. Rousset, 1995; GENEPOP version). General characteristics of the total study population, stratified by genotype, were compared by univariate ANOVA for continuous variables and \(\chi^2\) statistics for dichotomous variables. To examine the effect of the IGF-I genotype on IMT and PWV in hypertensive and normotensive subjects separately, we stratified all subjects on the basis of their genotype and the presence or absence of hypertension. Subjects homozygous for the 192-bp allele and normotensive were used as the reference category in these analyses. All analyses on systolic and diastolic blood pressure, IMT, and PWV were adjusted for possible confounders: age, sex, BMI, total cholesterol, HDL cholesterol, smoking, and diabetes mellitus. Additional analyses, adjusted for myocardial infarction and stroke, were performed to correct for possible confounding by the presence of prevalent cardiovascular disease. SPSS for Windows software package, version 10.0, was used to perform all analyses.

**TABLE 1. General Characteristics of Total Study Population Stratified by IGF-I Genotype**

<table>
<thead>
<tr>
<th></th>
<th>Homozygous Carriers</th>
<th>Heterozygous Carriers</th>
<th>Noncarriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>2240</td>
<td>2275</td>
<td>617</td>
</tr>
<tr>
<td>Serum total IGF-I levels, nmol/L*</td>
<td>20.5±6.2 (n=50)</td>
<td>19.6±6.4 (n=50)</td>
<td>16.7±5.0 (n=50)†</td>
</tr>
<tr>
<td>Men, %</td>
<td>43.3</td>
<td>44.2</td>
<td>40.2</td>
</tr>
<tr>
<td>Age, y</td>
<td>65.0±5.5</td>
<td>64.9±5.5</td>
<td>64.9±5.5</td>
</tr>
<tr>
<td>BMI, kg/m\textsuperscript{2}</td>
<td>26.3±3.7</td>
<td>26.2±3.5</td>
<td>26.4±3.6</td>
</tr>
<tr>
<td>Current smoking, %</td>
<td>25.7</td>
<td>26.8</td>
<td>21.4</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.7±1.2</td>
<td>6.7±1.2</td>
<td>6.7±1.1</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.4±0.4</td>
<td>1.3±0.4</td>
<td>1.4±0.4</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>8.2</td>
<td>8.4</td>
<td>11.9</td>
</tr>
</tbody>
</table>

All values are presented as mean±SD or percentage. 
*Measured in a subset of the total study population (n=150).
†Significantly different from homozygous carriers (\(P=0.003\).

<table>
<thead>
<tr>
<th>192-bp Allele</th>
<th>Homozygous Carriers</th>
<th>Heterozygous Carriers</th>
<th>Noncarriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure, mm Hg*</td>
<td>135.2±21.2</td>
<td>135.1±21.0</td>
<td>134.0±21.2</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg*</td>
<td>73.5±11.0</td>
<td>74.0±11.2</td>
<td>72.8±10.7</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>31.1</td>
<td>31.5</td>
<td>30.3</td>
</tr>
<tr>
<td>Intima-media thickness, mm</td>
<td>0.76±0.14</td>
<td>0.77±0.15</td>
<td>0.78±0.14†</td>
</tr>
<tr>
<td>Pulse wave velocity, m/s</td>
<td>13.3±2.9</td>
<td>13.3±3.0</td>
<td>13.3±2.9</td>
</tr>
</tbody>
</table>

All values are presented as mean±SD or percentage and adjusted for age, sex, BMI, total cholesterol, HDL cholesterol, diabetes, and smoking. *Only subjects without use of any blood pressure-lowering medication (n=3769). †Significantly different from homozygous carriers; P<0.05.

Results

Genotype frequencies in the total study population and in the normotensive and hypertensive subjects were in Hardy-Weinberg equilibrium (P=0.4).

In Table 1, the general characteristics of the total study population for each genotype group are presented. Serum total IGF-I levels were significantly lower in noncarriers of the 192-bp allele (16.7 nmol/L) compared with homozygous carriers (20.5 nmol/L) (P=0.003). Levels of cardiovascular risk factors are in the high-normal range, as expected in a population of elderly subjects. No significant differences between homozygous, heterozygous, and noncarriers of the 192-bp allele were observed. In Table 2, systolic and diastolic blood pressures, prevalence of hypertension, mean IMT of the carotid arteries, and aortic PWV are presented for each genotype group. Crude results (not shown) did not differ significantly from those after adjustment for possible confounders. Systolic and diastolic blood pressure and the prevalence of hypertension did not differ between the genotype groups. IMT was significantly increased in noncarriers of the 192-bp allele compared with heterozygous and homozygous carriers (P for trend=0.02). PWV did not differ between genotype groups in the overall analysis.

In Figure 1, mean IMT by genotype for all normotensive subjects, all hypertensive subjects, and untreated hypertensive subjects is presented. IMT was significantly higher in hypertensive subjects than in normotensive subjects in all genotype groups (P<0.005). In all hypertensive subjects, IMT was significantly higher in noncarriers of the 192-bp allele (0.83 mm) than in heterozygous and homozygous carriers (0.80 mm) (P=0.04). In untreated hypertensive subjects, IMT was also higher in noncarriers (0.85 mm) compared with heterozygous (0.80 mm) and homozygous carriers (0.80 mm) (P=0.01). In normotensive subjects, IMT did not differ significantly between the genotype groups. In Figure 2, mean PWV by genotype for all normotensive subjects, all hypertensive subjects, and untreated hypertensive subjects is presented. PWV was significantly higher in hypertensive subjects than in normotensive subjects in all genotype groups (P<0.005). In hypertensive subjects, PWV was significantly higher in noncarriers of the 192-bp allele (14.3 m/s) than in heterozygous carriers (14.1 m/s) and homozygous carriers (13.7 m/s) (P=0.02). In untreated hypertensive subjects, the difference in PWV between noncarriers and carriers of the 192-bp allele increased. Noncarriers had significantly higher PWV (15.0 m/s) than heterozygous (14.2 m/s) and homozygous carriers (13.8 m/s) (P=0.03). In normotensive subjects, PWV did not differ significantly between the genotype groups. Additional analyses with adjustment for myocardial infarction and stroke did not significantly change the results presented in Table 2 and Figures 1 and 2.

Discussion

In this population-based study we found an association between a genetic polymorphism in the promoter region of the IGF-I gene and IMT of the carotid arteries in the general population. Further analysis revealed that the association between this polymorphism and atherosclerosis was most

![Figure 1. IMT by IGF-I genotype in normotensive subjects, all hypertensive subjects, and untreated hypertensive subjects. Analysis was adjusted for age, sex, BMI, total cholesterol, HDL cholesterol, diabetes mellitus, and smoking. *P<0.05, significantly different from homozygous carriers in all hypertensive subjects. **P<0.01, significantly different from homozygous carriers in untreated hypertensive subjects.](image-url)
pronounced in hypertensive subjects. Hypertensive subjects who did not carry a copy of the 192-bp allele had an increased carotid IMT and higher aortic PWV than heterozygous and homozygous carriers of the 192-bp allele. The effect of this polymorphism was even stronger in subjects with untreated hypertension. In normotensive subjects, no association was found between this polymorphism and IMT or PWV.

Because IMT and PWV are considered reliable indicators of the structural and functional changes in the vasculature, we used both as early markers of atherosclerosis. Recent findings in our study population have shown a strong positive association between aortic stiffness (as measured by PWV) and common carotid IMT. PWV and IMT have been reported to be strongly associated with vascular risk factors and the prevalence of cerebrovascular and cardiovascular disease.

We have previously observed that noncarriers of the 192-bp allele have significantly lower circulating IGF-I levels than heterozygous or homozygous carriers of this allele. Findings by Janssen et al., who observed an inverse relation between circulating IGF-I levels and atherosclerosis in another population-based study, suggested that low IGF-I levels may play a role in the development of atherosclerosis in the general population.

Studies in growth hormone–deficient patients have already indicated that low circulating IGF-I levels may play an important role in the development of cardiovascular disease. Growth hormone–deficient patients have significantly increased IMT, decreased systemic nitric oxide generation, and a tendency toward impaired flow-mediated vasodilatation. They also have increased levels of circulating inflammatory cardiovascular risk markers, such as C-reactive protein, interleukin-6, and tumor necrosis factor-α. In addition, an increase in circulating IGF-I levels, observed during growth hormone replacement therapy, is accompanied by a reduction in IMT and a decrease in circulating inflammatory markers.

Although the exact mechanism by which IGF-I influences the development of atherosclerosis is still unknown, the effects of IGF-I on the vascular endothelium are thought to be partly mediated by nitric oxide, which not only induces vasorelaxation but also inhibits platelet aggregation, leukocyte adhesion, and smooth muscle cell growth.

Our findings support the observation that low levels of serum IGF-I may affect the development of atherosclerosis. Of interest is that the effect of this polymorphism on IMT and PWV was observed in hypertensive subjects only. One explanation for this finding may be that a relatively lower expression of the IGF-I gene in noncarriers of the 192-bp allele only becomes clinically relevant in subjects who have an increased demand for IGF-I. We hypothesize that in hypertensive subjects, because of increased hemodynamic load, more IGF-I is needed to protect the vessel wall than in normotensive subjects. Therefore, hypertensive subjects who do not carry a copy of the 192-bp allele may not have enough (reserve) capacity to adequately fulfill the increased demand for IGF-I. As a consequence, the antiatherogenic effects of IGF-I in these subjects may no longer be sufficient to prevent the development of atherosclerosis.

In our study we found an even stronger effect of this polymorphism in subjects with untreated hypertension. This observation supports our hypothesis that especially in subjects at high risk for atherosclerosis, as a result of increased hemodynamic load, the need for IGF-I is increased. In agreement with this is the observation in animal studies that IGF-I expression increases during periods of high hemodynamic load.

Since IGF-I is also known to induce vasorelaxation, it seems surprising that we did not observe an effect of this polymorphism in the IGF-I gene on blood pressure or the prevalence of hypertension. This suggests that IGF-I is not so much a determinant in the chronic blood pressure–lowering response but rather is a mediator in limiting the damaging effects of high blood pressure on the vasculature.

Another important issue is possible confounding by other cardiovascular risk factors such as diabetes mellitus, impaired glucose tolerance, increased BMI, hyperlipidemia, and preexisting cardiovascular disease. These risk factors have also been associated with low IGF-I levels. However, our results did not change significantly after adjustment for these risk factors, suggesting that the effects of the IGF-I poly-
morphism on the vascular endothelium are independent of other cardiovascular risk factors.

In conclusion, our findings support the opinion that IGF-I plays a role in the pathogenesis of atherosclerosis. The polymorphism we studied in the IGF-I gene most likely is a modifier of the risk for atherosclerosis in subjects with hypertension. Ongoing studies on the role of IGF-I and the interaction with its receptor and binding proteins will help to further elucidate the exact mechanism by which IGF-I exerts its effects on the development of atherosclerosis in subjects with high blood pressure.

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