

# A Polymorphism in the Gene for IGF-I

## Functional Properties and Risk for Type 2 Diabetes and Myocardial Infarction

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Evidence is accumulating that low levels of IGF-I play a role in the pathogenesis of type 2 diabetes and cardiovascular diseases. We examined the role of a genetic polymorphism in the promoter region of the IGF-I gene in relation to circulating IGF-I levels and growth measured as body height, and we studied the relationship of this polymorphism with type 2 diabetes and myocardial infarction. The relation between the IGF-I polymorphism and body height was assessed in a population-based sample of 900 subjects from the Rotterdam Study. Within each genotype stratum, 50 subjects were randomly selected for a study of the relation of this polymorphism with serum IGF-I levels. To assess the risk for type 2 diabetes, we studied 220 patients and 596 normoglycemic control subjects. For myocardial infarction, 477 patients with evidence of myocardial infarction on electrocardiogram and 808 control subjects were studied. A 192-bp allele was present in 88% of the population, suggesting that this is the wild-type allele from which all other alleles originated. Body height was, on average, 2.7 cm lower (95% CI for difference -4.6 to -0.8 cm,  $P = 0.004$ ), and serum IGF-I concentrations were 18% lower (95% CI for difference -6.0 to -1.3 mmol/l,  $P = 0.003$ ) in subjects who did not carry the 192-bp allele. In noncarriers of the 192-bp allele, an increased relative risk for type 2 diabetes (1.7 [95% CI 1.1-2.7]) and for myocardial infarction (1.7 [95% CI 1.1-2.5]) was found. In patients with type 2 diabetes, the relative risk for myocardial infarction in subjects without the 192-bp allele was 3.4 (95% CI 1.1-11.3). Our study suggests that a genetically determined exposure to relatively low IGF-I levels is associated with an increased risk for type 2 diabetes and myocardial infarction. *Diabetes* 50:637-642, 2001

Insulin-like growth factor I (IGF-I) is a peptide that stimulates bone growth, cell differentiation, and metabolism. The structural and functional homologies with insulin (1), as well as the hypoglycemic insulin-like effects observed after the administration of recombinant IGF-I, suggest that this peptide is involved in the regulation of glucose homeostasis (2). In patients with type 2 diabetes, low serum IGF-I levels are common (3,4). Evidence is accumulating that these low IGF-I levels may play a role in the development of the vascular complications of type 2 diabetes (5,6). IGF-I may also play a role in the regulation of cardiovascular function (7,8) and development of myocardial infarction in subjects without type 2 diabetes, although various studies have yielded conflicting results regarding the direction of the association between circulating IGF-I levels and cardiovascular disease (9-14). Studies of the role of IGF-I in the development of disease have been hampered by the fact that circulating IGF-I levels do not necessarily reflect the local production of IGF-I in specific tissues, such as the myocardium or pancreatic  $\beta$ -cells. A genetic polymorphism in the IGF-I gene promoter region has been identified, which may influence IGF-I production (15,16). This may create an opportunity to characterize, on a genetic basis, subjects who are chronically exposed to low IGF-I levels throughout the body. Until now, studies of this polymorphism in relation to IGF-I levels and pathology have been limited to patients with osteoporosis and related disorders (16-18).

We examined the role of a known genetic polymorphism in the promoter region of the IGF-I gene in relation to circulating IGF-I levels and growth measured as body height, and we studied the relationship of this polymorphism with type 2 diabetes and myocardial infarction.

### RESEARCH DESIGN AND METHODS

**Subjects.** This study is part of the Rotterdam Study, a single-center prospective follow-up study in which all residents of the Rotterdam suburb Ommoord aged 55 years and older were invited to take part. The study was approved by the Medical Ethics Committee of Erasmus Medical Center Rotterdam (the Netherlands), and written informed consent was obtained from all participants. The aim of the study was to investigate determinants of chronic and disabling cardiovascular, neurodegenerative, locomotor, and ophthalmologic diseases. The design of the study has been described previously (19). The baseline examination of the Rotterdam Study, on which this report is based, was conducted between 1990 and 1993. A total of 7,983 participants (response

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ECG, electrocardiogram; PCR, polymerase chain reaction; WHR, waist-to-hip ratio.

TABLE 1  
Baseline characteristics of the study populations

	Population sample	Diabetes study		Myocardial infarction study	
		Case subjects	Control subjects	Case subjects	Control subjects
<i>n</i>	900	220	596	477	808
Age (years)	65.9 (5.6)	68.2 (5.6)†	66.2 (5.4)	66.5 (5.2)*	65.7 (5.6)
Men	39.9%	56.4%*	47.3%	69.2%†	38.6%
Current smokers	25.3%	20.1%	24.1%	26.5%	25.2%
BMI (kg/m <sup>2</sup> )	26.6 (3.8)	27.6 (3.5)*	25.9 (3.2)	26.6 (3.8)	26.6 (3.6)
WHR	0.90 (0.09)	0.94 (0.09)†	0.89 (0.09)	0.94 (0.08)†	0.90 (0.09)
Total cholesterol level (mmol/l)	6.8 (1.2)	6.4 (1.2)	6.6 (1.1)	6.6 (1.2)	6.8 (1.2)
HDL cholesterol level (mmol/l)	1.4 (0.3)	1.2 (0.3)†	1.4 (0.3)	1.2 (0.3)†	1.4 (0.3)
Hypertension	38.6%	55.7%†	31.7%	59.1%†	36.6%

Data are means (SD) or percentages. \**P* = 0.05; †*P* = 0.005.

rate 78%) were examined. In the present study, we only included subjects between 55 and 75 years of age at the time of baseline examination.

We followed a five-phase approach to study the relation of a known cytosine-adenine repeat in the promoter region of the human IGF-I gene (15) with growth, IGF-I serum levels, and risk for type 2 diabetes and cardiovascular disease. In phase 1, we selected a population-based sample of 900 participants to assess genotype and allele frequencies of the polymorphism and their relationship with body height. The 900 subjects were randomly drawn from all participants in the age groups of 55–65 years and 65–75 years and were frequency matched for the age distribution of the myocardial infarction cases (described later). The number of subjects was based on power calculations to detect a difference in body-height of at least 1.5 cm with a power of 80% and a significance level of 0.05. In phase 2, 50 subjects were randomly drawn from each genotype stratum in the population-based sample. In these subjects, the relation between the promoter polymorphism and serum IGF-I concentration was studied. The selection of these 50 subjects per genotype was based on power calculations to detect a difference of at least 15% in serum IGF-I concentration between genotypes with a power of 80% and a significance level of 0.05. In phase 3, the association of the polymorphism with type 2 diabetes was assessed in a case-control study. For practical and financial reasons, only a proportion of the Rotterdam Study (*n* = 1,110) underwent a fasting oral glucose tolerance test. From this group, we selected our case and control subjects. Included were patients being treated for type 2 diabetes and patients in whom type 2 diabetes had been diagnosed recently, based on a fasting glucose level of 7.0 mmol/l or higher and/or a 2-h postload glucose measurement of 11.1 mmol/l or higher after a fasting 75-g oral glucose tolerance test (20). The 220 patients identified were compared with 596 normoglycemic control subjects. Normoglycemia was defined as a fasting glucose level lower than 6.1 mmol/l and a 2-h postload glucose sample lower than 7.8 mmol/l.

In phases 4 and 5, the association of the polymorphism with myocardial infarction was assessed. In phase 4, 477 participants with myocardial infarction confirmed on electrocardiogram (ECG) at the baseline examination were compared with 808 control subjects who had no evidence of myocardial infarction on ECG. These control subjects were selected from the age-frequency-matched population of 900 subjects of phase 1. In all participants, a resting standard 12-lead ECG was recorded with an ACTA Gnosis IV (EsaoteBiomedica) (21,22). To diagnose myocardial infarction, an automated diagnostic classification system, the Modular ECG Analysis System (MEANS) (21,22), was used. Infarctions detected without evidence of symptoms (silent myocardial infarctions) were verified by an experienced cardiologist (21,22). Finally, in phase 5, the relative risk for myocardial infarction in the 220 subjects with type 2 diabetes was studied in relation to the IGF-I promoter polymorphism using the clinical criteria described above.

**Measurements.** At the baseline examination, information concerning health status, drug use, and smoking behavior was obtained using a computerized questionnaire. Height and weight were measured and BMI (kg/m<sup>2</sup>) was calculated. Body fat distribution was assessed by the waist-to-hip ratio (WHR). Blood pressure was measured in sitting position at the right upper arm with a random-zero sphygmomanometer, and the average of two measurements obtained at one occasion was used. Hypertension was defined as a diastolic blood pressure of 95 mmHg or higher and/or a systolic blood pressure of 160 or higher and/or the use of antihypertensive drugs.

Blood sampling and storage have been described elsewhere (23). Serum was separated by centrifugation and quickly frozen in liquid nitrogen. Baseline measurements were performed on nonfasting blood samples. Total serum cholesterol and HDL cholesterol levels were determined using an automated

enzymatic procedure (24). Glucose levels were measured by the glucose hexokinase method in fasting serum and postload serum samples (25). Total IGF-I was determined by a commercially available radioimmunoassay (Medgenix Diagnostics) with intra-assay and interassay variation of 6.1 and 9.9%, respectively, in nonfasting serum.

Polymerase chain reaction (PCR) was performed using oligonucleotide primers designed to amplify the polymorphic cytosine-adenine repeat 1 kb upstream of the human IGF-I gene (15). The reaction was carried out in a final volume of 10  $\mu$ l containing 50 ng of genomic DNA obtained from peripheral white blood cells, 0.5 nmol/l forward primer (5'-ACCACTCTGGGAGAAG GGTA-3'), 0.5 nmol/l reverse primer (5'-GCTAGCCAGCTGGTGTATT-3'), 0.25 mmol/l 2'-dNTP, 2.2 mmol/l MgCl<sub>2</sub>, 0.01% W1 (Gibco BRL), and 0.4 U *Taq* DNA polymerase (Gibco BRL). PCR was performed in 384 well plates (94°C 10 min; 35 PCR cycles of 30 s at 94°C, 30 s on 55°C, and 30 s on 72°C; 72°C 10 min; 4°C hold). Forward primers were labeled with FAM, HEX, or NED to determine the size of PCR products by autosequencer (ABI 377, 6.25% longranger gel, filter set D, peak height between 100 and 2,000, each lane containing three samples). The size of the PCR products was determined in comparison with internal ROX 500-size standard (Perkin Elmer).

**Statistical analysis.** Hardy-Weinberg equilibrium of the IGF-I promoter polymorphism genotypes was tested with the GENESOP-package (26). Body height and serum IGF-I concentration were compared between genotypes using analyses of variance. Body height was adjusted for the possible confounders age and sex. Participants who were taking medications for type 2 diabetes or hormone conditions were excluded from the analyses of serum IGF-I, because these types of medication are known to influence IGF-I concentrations (27–29). Furthermore, serum IGF-I was adjusted for the possible confounders age, sex, and BMI. Serum IGF-I values were logarithmically transformed for the analyses. The nontransformed data and standard errors are presented for both body height and IGF-I.

A multiple logistic regression model was used to study the association of the IGF-I promoter polymorphism genotypes with type 2 diabetes and myocardial infarction. Each patient group was compared with the specific control group selected. Relative risks were estimated as odds ratios and presented with a 95% CI. Both crude relative risks and relative risks after adjustment for the possible confounders age, sex, body height, BMI, WHR, total cholesterol level, HDL cholesterol level, and hypertension are presented in Table 1. All analyses were performed using the SPSS for Windows (version 7.5.2) software package.

## RESULTS

The characteristics of the participants in the studies are presented in Table 1. In both the myocardial infarction study and type 2 diabetes study, case subjects were slightly older and included more men. BMI was significantly increased in patients with type 2 diabetes, whereas the WHR was significantly increased both in patients with type 2 diabetes and patients with myocardial infarction. Low HDL cholesterol levels and hypertension were frequently seen in both case groups. In the population-based sample of 900 subjects, 10 different alleles were identified in the promoter polymorphism of the IGF-I gene (Table 2). Genotype and allele distributions were in Hardy-Weinberg

TABLE 2  
Allele distribution of the IGF-I promoter polymorphism

Allele (no. of base pairs)	Male ( <i>n</i> = 720)	Female ( <i>n</i> = 1,080)	Total ( <i>n</i> = 1,800)
200	1 (0.1)	—	1 (0.1)
198	9 (1.3)	16 (1.5)	25 (1.4)
196	44 (6.1)	80 (7.4)	124 (6.9)
194	118 (16.4)	202 (18.7)	320 (17.8)
192	505 (70.1)	712 (65.9)	1,217 (67.6)
190	26 (3.6)	44 (4.1)	70 (3.9)
188	15 (2.1)	21 (1.9)	36 (2.0)
186	2 (0.3)	3 (0.3)	5 (0.3)
184	—	2 (0.2)	2 (0.1)
182	1 (0.1)	—	1 (0.1)

Data are *n* (%). \*Allele distribution based on a population-based sample of 900 participants (1,800 alleles: 2 alleles per subject).

equilibrium ( $P = 0.76$ ). When considering genotypes, 88.4% of the subjects from the population-based sample were homozygous or heterozygous for a 192-bp allele, suggesting that this is the wild-type allele from which all other alleles originated. The frequency of the other nine alleles was low; therefore, these alleles were pooled in the analyses. This resulted in three possible genotypes: subjects who were homozygous for the 192-bp allele (46.7%), subjects who were heterozygous for the 192-bp allele (41.7%), and noncarriers of the 192-bp allele (11.6%).

In the population-based sample of 900 subjects, body height increased with the number of 192-bp alleles present ( $P$  for trend = 0.01, Fig. 1A). Mean body height was significantly lower in noncarriers of the 192-bp allele (165.4 cm) compared with subjects who were homozygous for the 192-bp allele (168.1 cm [95% CI for difference -4.6 to -0.8 cm,  $P = 0.004$ ]). Also, the mean serum IGF-I increased with the number of 192-bp alleles carried ( $P$  for trend = 0.003, Fig. 1B). In noncarriers of the 192-bp allele, the mean serum total IGF-I concentration was 18% lower (16.7 mmol/l) compared with subjects who were homozygous for the 192-bp allele (20.5 mmol/l [95% CI for difference -6.0 to -1.3 mmol/l,  $P = 0.003$ ]). The findings related to body height and IGF-I levels could not be attributed to a specific allele of the IGF-I promoter polymorphism.

As demonstrated in Table 3, the IGF-I genotype is not associated with previously identified correlates of myocardial infarction or type 2 diabetes. As shown in Table 4, fewer subjects with type 2 diabetes or myocardial infarction were homozygous for the 192-bp allele compared with the control groups. Compared with subjects who were homozygous for the 192-bp allele, noncarriers of the 192-bp allele had a relative risk of 1.7 for developing type 2 diabetes (95% CI 1.1–2.7) as well as for myocardial infarction (95% CI 1.1–2.5). For subjects who were heterozygous for the 192-bp allele, the relative risk was 1.4 for developing type 2 diabetes (95% CI 1.0–1.9) and 1.2 for developing myocardial infarction (95% CI 0.9–1.5). Although the IGF-I polymorphism was strongly associated with body height, no statistically significant association between body height and type 2 diabetes or myocardial infarction was found. However, comparing body height with control subjects, on average, patients with type 2 diabetes were 0.6 cm shorter ( $P = 0.23$ ) and patients with myocardial infarction were 0.4 cm shorter ( $P = 0.29$ ).

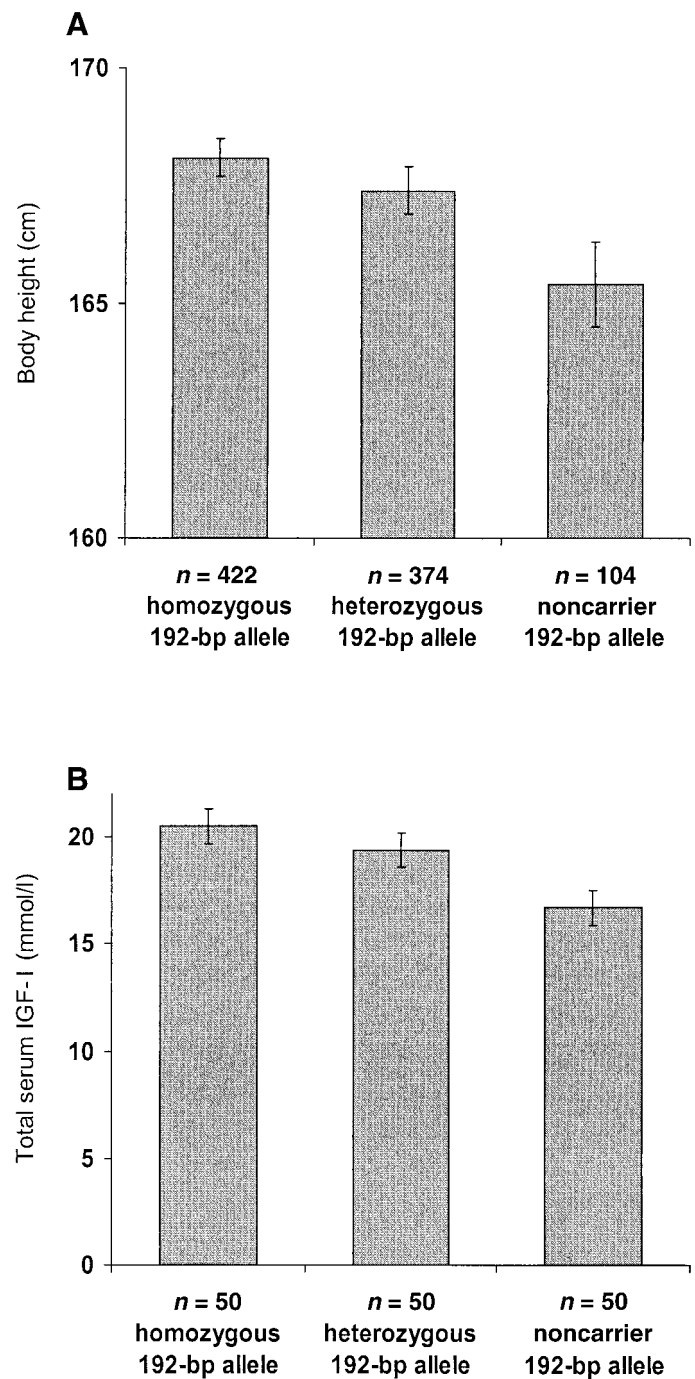


FIG. 1. Body height and serum IGF-I level in relation to the IGF-I promoter polymorphism. A: Body height as mean with standard error. B: Mean serum IGF-I concentration with standard error (mmol/l).

Correction for these small differences in body height in our analysis did not change the risk estimates for type 2 diabetes or myocardial infarction associated with the IGF-I genotype. Within the group of patients with type 2 diabetes, 34 subjects with myocardial infarction were identified. The prevalence of myocardial infarction was 25% in subjects without the 192-bp allele (Fig. 2). In noncarriers of the 192-bp allele, the relative risk for myocardial infarction was 3.4 (95% CI 1.1–11.3), whereas for subjects who were heterozygous for the 192-bp allele,

TABLE 3

Relation between IGF-I promoter genotype and previously identified correlates of type 2 diabetes or myocardial infarction

	Homozygous 192-bp allele	Heterozygous 192-bp allele	Noncarrier 192-bp allele	<i>P</i>
Age (years)	66.4 (5.4)	65.4 (5.6)	66.1 (6.0)	0.11
Men	179 (42.4%)	147 (39.2%)	34 (32.7%)	0.18
Current smokers	98 (23.4%)	108 (29.0%)	21 (20.6%)	0.30
BMI (kg/m <sup>2</sup> )	26.6 (3.9)	26.8 (3.8)	26.5 (3.0)	0.71
WHR	0.90 (0.09)	0.91 (0.09)	0.90 (0.1)	0.52
Total cholesterol level (mmol/l)	6.8 (1.3)	6.8 (1.2)	6.7 (0.9)	0.93
HDL cholesterol level (mmol/l)	1.3 (0.4)	1.4 (0.4)	1.4 (0.3)	0.27
Hypertension	170 (40.5%)	140 (37.3%)	37 (35.6%)	0.53

Data are means (SD) or *n* (%). Measurements based on population sample of 900 subjects.

the relative risk for myocardial infarction was 2.9 (95% CI 1.1–7.7).

### DISCUSSION

In this population-based study, we found that the absence of the wild-type (192-bp) allele of a genetic polymorphism in the regulatory region of the IGF-I gene is significantly associated with low body height and low serum levels of IGF-I. The main finding of the study is that the absence of this allele is also significantly associated with an increased risk for type 2 diabetes and myocardial infarction. Particularly in subjects with type 2 diabetes, the relative risk for myocardial infarction is strongly increased in subjects who are noncarriers of the 192-bp allele.

The polymorphism under study is a cytosine-adenosine repeat 1 kb upstream from the transcription site of the IGF-I gene (30). Given the population-based approach of our study, we cannot distinguish whether this polymorphism itself is involved in regulation of IGF-I expression or merely flags another polymorphism in the promoter region functionally involved in IGF-I expression. However, the association with low body height and low IGF-I serum level suggests that the absence of the 192-bp allele characterizes subjects who are chronically exposed to low IGF-I levels throughout the body. Using this polymorphism, the difficulty in measuring local IGF-I production in relevant tissues, such as the myocardium or the pancreatic  $\beta$ -cell, can be circumvented. Furthermore, this genetic approach overcomes the problem that cross-sectional studies cannot distinguish whether changes in IGF-I levels are a cause or rather a consequence of disease.

In our population-based sample from the Rotterdam Study, the 192-bp allele was present in 88% of subjects. This finding is in agreement with other reports in Caucasian populations (16,17). In contrast with a previous report

by Rosen et al. (16), subjects who were homozygous for the 192-bp allele had significantly higher serum levels of IGF-I compared with subjects who were noncarriers of this allele. However, the study by Rosen et al. was based on a relatively small, highly selected study population that included patients with chronic chest pain, patients with idiopathic osteoporosis, participants of a calcium intervention trial, and participants of a study on body mass (16). The inclusion of patients with putative IGF-I-related pathology might explain the discrepancy with the findings in our population-based study. Our observation of high IGF-I concentrations in subjects who were homozygous for the 192-bp allele are supported by the fact that these subjects were also significantly taller by nearly 3 cm compared with subjects who were noncarriers of the 192-bp allele.

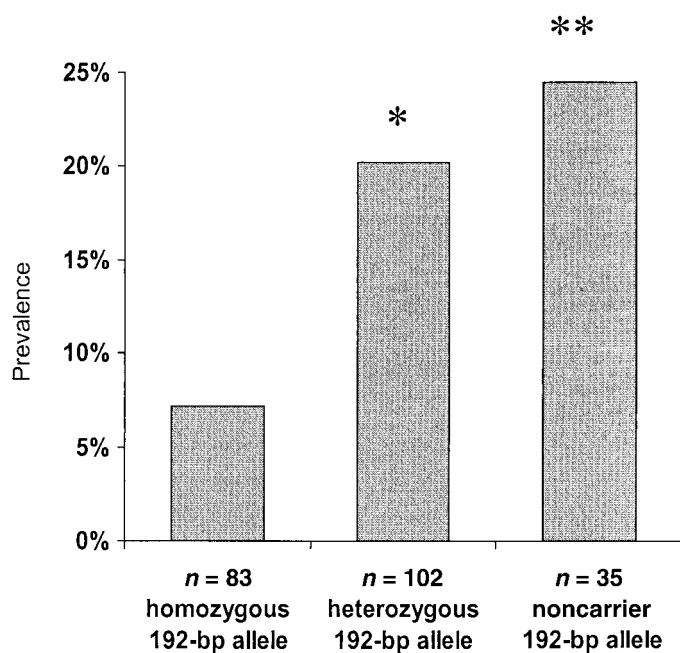
This is the first study of the role of the IGF-I promoter polymorphism in the pathogenesis of type 2 diabetes and cardiovascular disease. The increased relative risk for type 2 diabetes and myocardial infarction in noncarriers of the 192-bp allele suggests that a lifetime exposure to moderate alterations in IGF-I expression may also be biologically relevant in terms of disease risk. These observations are in accordance with earlier reports of low-normal circulating IGF-I levels in patients with cardiovascular disease (31) and type 2 diabetes (3). Considering the association with both myocardial infarction and type 2 diabetes, this IGF-I promoter polymorphism may explain, to some extent, the clustering of these diseases. The polymorphism is a particularly strong predictor of myocardial infarction in subjects with type 2 diabetes. If these findings can be replicated by others, this may present an opportunity to identify patients with type 2 diabetes who are at high risk for myocardial infarction and who may benefit from specific therapy influencing the IGF-I metabolism. Further

TABLE 4

Relative risk of type 2 diabetes and myocardial infarction based on the presence of the 192-bp allele

	Homozygous 192-bp allele	Heterozygous 192-bp allele	Noncarrier 192-bp allele
Type 2 diabetes	83 (37.7%)	102 (46.4%)	35 (15.9%)
Control subjects	277 (46.5%)	248 (41.6%)	71 (11.9%)
Crude relative risk	Reference	1.4 (1.0–1.9)	1.6 (1.0–2.6)
Adjusted relative risk*	Reference	1.4 (1.0–1.9)	1.7 (1.1–2.7)
Myocardial infarction	205 (43.0%)	204 (42.8%)	68 (14.3%)
Control subjects	383 (47.4%)	340 (42.1%)	85 (10.5%)
Crude relative risk	Reference	1.1 (0.9–1.4)	1.5 (1.0–2.1)
Adjusted relative risk*	Reference	1.2 (0.9–1.5)	1.7 (1.1–2.5)

Data are number of subjects for each category (% of case subjects or control subjects). Risks are given with a 95% CI. \*Adjustment for the possible confounders age, sex, WHR, BMI, total cholesterol level, HDL cholesterol level, and hypertension.



**FIG. 2.** Prevalence of myocardial infarction in 220 patients with type 2 diabetes. \*Significantly different from subjects homozygous for the 192-bp allele ( $P < 0.01$ ). \*\*Significantly different from subjects homozygous for the 192-bp allele ( $P < 0.05$ ).

studies are needed for a better understanding of the specific pathogenic pathways involved.

Our population-based study suggests that a genetic polymorphism in the promoter region of the IGF-I gene is associated with IGF-I expression. The absence of a 192-bp allele of this polymorphism, as observed in almost 12% of the general population, is associated with lower body height, low IGF-I levels, and an increased risk for type 2 diabetes and myocardial infarction. The increased risk for these disorders is most likely a result of a genetically determined, lifelong exposure to relatively low IGF-I concentrations in subjects who are noncarriers of the 192-bp allele.

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