The role of IGF-I in the pathogenesis of diabetic retinopathy is unclear. We studied, prospectively, the relationship between an IGF-I gene polymorphism, retinal vessel diameters, and incident diabetic retinopathy in subjects with impaired glucose tolerance (IGT) or type 2 diabetes. In all 5,505 participants of the population-based Rotterdam Study (775 with IGT, 394 with type 2 diabetes, and 4,336 control subjects), fundus color transparencies were taken at baseline (between 1990 and 1993) and at follow-up (from 1997 to 1999). The wild-type genotype (i.e., carriers of the 192- or 194-bp alleles) was present in 72.7% of the participants, while 27.3% were variant carriers. Variant carriers with IGT or type 2 diabetes appeared to have larger retinal arteriolar and venular diameters at baseline than individuals with the wild-type genotype, but these differences did not reach statistical significance. This trend was especially observed in subjects who developed retinopathy at follow-up. In variant carriers with IGT/diabetes, an increase (odds ratio 1.8 [95% CI 1.0–3.2]; \( P = 0.04 \)) in the risk of retinopathy was observed compared with participants with the wild-type genotype. In conclusion, our findings suggest that this IGF-I gene polymorphism is associated with an increased risk of diabetic retinopathy.

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The role of IGF-I in the pathogenesis of diabetic retinopathy (hereafter referred to simply as retinopathy) in both type 1 and type 2 diabetes is unclear and controversial. It has been suggested that hereditary factors are involved in the pathogenesis to develop retinopathy (1).

In humans, a Ca\(_{n}\) polymorphism in the promoter region of the IGF-I gene has been identified (2). We previously observed that this Ca\(_{n}\) polymorphism indeed was associated with low normal serum total IGF-I levels, a lower body height, and a higher risk for developing type 2 diabetes and myocardial infarction (2). Although these findings have not been consistently confirmed in other studies, they suggest that this IGF-I gene polymorphism may be used as a proxy for the genetically determined IGF-I expression in the body.

The purpose of our study was to examine the associations between this Ca\(_{n}\) IGF-I gene promoter polymorphism and retinal vascular diameters, as well as diabetic retinopathy, in participants with type 2 diabetes and impaired glucose tolerance (IGT).

**RESEARCH DESIGN AND METHODS**

The Rotterdam Study is a single-center prospective follow-up study in which all residents aged ≥55 years from a suburb of Rotterdam were invited to participate (3). The appropriate medical ethics committees of the Erasmus University approved the study, and written informed consent was obtained from all participants. In total, 7,983 participants (response rate 78%) were examined.

The ophthalmologic part of the Rotterdam Study became operational after the screening of the participants had started, leading to ophthalmologic examinations of 6,780 individuals. Of them, 6,436 had transparencies taken. From these, 762 were excluded because they had undiagnosed fundus transparencies on either eye, resulting in 5,674 eligible individuals (4). Glucose was measured in 5,505 of the 5,674 individuals of the ophthalmologic cohort. Genotyping for the IGF-I gene polymorphism was successful for 5,393 of the 5,505 individuals whose information on glucose tolerance was available. The present study was based on these 5,393 individuals (4,247 control subjects, 759 subjects with IGT, and 387 type 2 diabetic subjects).

**Ophthalmologic examination.** Baseline examinations were conducted between 1990 and 1993, and for the prospective study, we used data from the follow-up period of 1997–1999 (n = 3,296 participants [554 individuals with IGT/diabetes] and mean follow-up time 6.5 years [range 5.1–8.5]). At baseline and follow-up, 35° color fundus transparencies, centered on the fovea (field 2), were taken after pharmacological mydriasis (4).

To measure the sum of the retinal vessel diameters, 20° field color transparencies taken at baseline and centered on the optic disc were digitized (4). Per person, the image with the best quality (left or right eye) was analyzed with a semiautomated system (Retinal Analysis; Optimate, Madison, WI) by four trained graders masked for any end point (5–7). Summary retinal arteriolar and venular diameters (in micrometers) were calculated with the improved Parr-Hubbard formula, adjusted for magnification differences due to possible refractive errors of the eye (5,8). In a random subsample of 100 participants, we found no differences between the right and left eyes for the arteriolar and venular diameters; therefore, only one eye of each individual was used.

For the prospective study, the level of retinopathy was graded in each eye according to the Early Treatment Diabetic Retinopathy Study scale (9,10). Grading was performed at baseline and at follow-up. Incident retinopathy was defined as absent retinopathy at baseline and presence of retinopathy in any eye at follow-up.

**Other measurements.** Information concerning health and smoking status was obtained during a home interview at baseline. Blood pressure was measured with a random zero sphygmomanometer. Hypertension was defined as a diastolic blood pressure ≥90 mmHg and/or a systolic blood pressure ≥140 mmHg and/or the use of antihypertensive medication (11). Blood
were performed using the SPSS for Windows software package, version 11.0.

Mean arteriolar and venular diameters at baseline were not different between participants with the wild type (n = 3,922) and variant carriers (n = 1,471) (arteriolar diameter [means ± SE] in participants with the wild type 146.7 ± 0.2 μm vs. variant carriers 147.2 ± 0.4 μm, P = 0.3; venular diameters in individuals with the wild type 221.7 ± 0.3 μm vs. variant carriers 222.5 ± 0.5 μm, P = 0.2).

Variant carriers with IGT or type 2 diabetes appeared to have larger retinal arteriolar and venular diameters at baseline than participants with the wild type, but these differences did not reach statistical significance (Table 2).

When further separated in those with and without incident retinopathy during follow-up, mean retinal arteriolar and venular diameters at baseline tended to be larger in variant carriers with incident retinopathy during follow-up than in those without (Table 3), but probably due to the low numbers, these differences did not reach statistical significance (Table 3).

Retinal arteriolar and venular diameters at baseline tended to be the largest in variant carriers with IGT or type 2 diabetes, who developed incident retinopathy during follow-up, compared with control subjects and variant carriers with IGT or type 2 diabetes, who did not develop retinopathy (retinal arteriolar diameter P for trend = 0.03 and retinal venular diameter P for trend = 0.02; Figs. 1 and 2). For participants with the wild type with IGT or type 2 diabetes, such trend was not observed (Figs. 1 and 2).

In the group with IGT or type 2 diabetes, variant carriers had an increased risk (OR 1.8 [95% CI 1.0–3.2]; P = 0.04) (adjusted for age and sex) of incident retinopathy compared with participants with the wild type.

### RESULTS

At baseline, participants with IGT or type 2 diabetes were younger, were more frequently male, had a higher systolic blood pressure, were more often diagnosed with hypertension, and were more frequently current smokers than the control subjects (Table 1). They also had significantly higher mean retinal arteriolar and venular diameters than control subjects (Table 1).

### TABLE 2

Differences in baseline characteristics and ophthalmologic parameters in participants with IGT/diabetes comparing subjects with the wild type and variant carriers of an IGF-I gene polymorphism

| IGT/diabetes | Wild type | Variant carriers | P value* |
|--------------|-----------|-----------------|---------|
| n            | 824 (71.9)| 322 (28.1)      | 0.3     |
| Age (years)  | 69.4 ± 0.3| 69.9 ± 0.5      | 0.3     |
| Male         | 403 (48.9)| 146 (45.3)      | 0.3     |
| Systolic blood pressure (mmHg) | 142.9 ± 0.8 | 142.8 ± 1.2 | 0.9     |
| Diastolic blood pressure (mmHg) | 73.6 ± 0.4 | 73.5 ± 0.7 | 0.9     |
| Hypertension | 353 (42.8)| 142 (44.1)      | 0.4     |
| Current smoker | 219 (26.6)| 79 (24.5)       | 0.7     |
| Random glucose (mmol/l) | 10.4 ± 0.2 | 10.5 ± 0.2 | 0.8     |
| Arteriolar diameter (μm) | 146.9 ± 0.5 | 148.5 ± 0.8 | 0.07    |
| Venular diameter (μm) | 222.6 ± 0.7 | 224.7 ± 1.2 | 0.1     |

Data are means ± SE or n (%). *After adjustment for age and sex.
DISCUSSION

In participants with IGT or diabetes, the risk of retinopathy was significantly higher in variant carriers than in subjects with the wild-type genotype of this IGF-I gene promoter polymorphism. Thus, this suggests that variant carriers have an increased risk of retinopathy and opens the option that IGF-I gene variants are risk factors and/or markers for phenotypes related to overall IGF-I expression (14). The IGF-I gene variant of an individual is fixed for life, and the relationship between an IGF-I variant and a phenotype is not susceptible to potential confounding factors such as age, insulin deficiency, hyperglycemia, and nutrition state. However, it is at present unknown whether this IGF-I gene polymorphism directly affects expression of IGF-I in vivo and mediates its effects through circulating IGF-I levels. No in vitro studies have been carried out demonstrating that this IGF-I gene promoter polymorphism is indeed associated with modified IGF-I gene expression. Variations in gene variants are traditionally difficult to study; subtle differences between gene variants may be present that are not detected by the presently available in vitro assays (15,16). Another unanswered question at the moment is whether this IGF-I gene promoter polymorphism is related to paracrine/autocrine IGF-I production in the body. This latter aspect could be very important because it is not only thought that the IGF-I system has important paracrine/autocrine actions but also that locally produced IGF-I may produce effects other than circulating IGF-I (17).

Although these differences did not reach statistical significance, variant carriers with IGT or diabetes who developed retinopathy at follow-up tended to have the largest retinal arteriolar and venular diameters (Figs. 1B and 2B). In contrast, such a trend was absent in participants with the wild type with IGT or diabetes who developed retinopathy at follow-up (Figs. 1A and 2A). Vascular dilatation of the retinal vessels, especially the retinal venules, is observed in the early stages of diabetic retinopathy.

TABLE 3

|                          | Wild type                          | Variant carriers                   | P value* |
|--------------------------|------------------------------------|------------------------------------|----------|
| Mean retinal arteriolar diameter (μm) |                                    |                                    |          |
| Participants with IGT/diabetes |                                    |                                    |          |
| Without incident retinopathy | 147.8 ± 0.8 (361)                 | 148.2 ± 1.2 (136)                 | 0.45     |
| With incident retinopathy    | 151.1 ± 2.7 (31)                   | 153.1 ± 3.7 (18)                  | 0.54     |
| Mean retinal venular diameter (μm) |                                    |                                    |          |
| Participants with IGT/diabetes |                                    |                                    |          |
| Without retinopathy       | 224.6 ± 1.1 (361)                 | 226.4 ± 1.8 (136)                 | 0.39     |
| With retinopathy         | 222.7 ± 3.8 (31)                   | 230.8 ± 4.6 (18)                  | 0.20     |

Data are means ± SE (n). For the analysis, the two genotypes were further separated in those with and without incident retinopathy. *After adjustment for age and sex.

FIG. 1. Mean (±SE) baseline retinal arteriolar diameters comparing control subjects, participants with IGT/diabetes without retinopathy (IGT/DM RP-), and participants with IGT/diabetes with retinopathy (IGT/DM RP+) in subjects with the wild type (A) and variant carriers (B). *After adjustment for age and sex.
retinopathy (18). Vascular dilatation is thought to indicate increased retinal blood flow and is probably related to hyperglycemic hypoxia and reduced vascular tone (18). In addition, larger arteriolar and venular diameters, independent of retinopathy severity level, have been associated with increased 4-year progression of retinopathy in type 1 diabetes (19,20). Moreover, larger venular diameters were positively associated with 4-year incidence of proliferative retinopathy (19). Our study observed larger retinal diameters at baseline in variant carriers with IGT or diabetes, who developed incident retinopathy at follow-up, and the absence of this trend in participants with the wild type may thus already point out at baseline an increased risk for progression of retinopathy in variant carriers than in participants with the wild type.

The low observed incidence of retinopathy in our study is probably related to the fact that we studied only field 2 of the standard diabetic fundus photographs. By studying only this field, we had no information on ~50% of the retina, and this will have contributed to an underestimation of the incidence of retinopathy (R. Klein, personal communication).

In conclusion, in individuals with IGT or diabetes, variant carriers of this IGF-I gene polymorphism had an increased risk of retinopathy compared with carriers of the wild-type genotype during a mean follow-up of 6.5 years. Variant carriers with IGT/diabetes, who developed retinopathy during follow-up, had a significant trend for larger retinal arteriolar and venular diameters at baseline, while such trend was absent for subjects with the wild-type genotype with IGT or diabetes, who developed retinopathy at follow-up. Since larger arteriolar and venular diameters have been associated with an increased progression of retinopathy, our findings suggest that this IGF-I gene polymorphism may modulate the susceptibility and/or the progression of retinopathy.

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