Genetic Factors and Insulin Secretion

Gene Variants in the IGF Genes

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IGFs are important regulators of pancreatic β-cell development, growth, and maintenance. Mutations in the IGF genes have been found to be associated with type 2 diabetes, myocardial infarction, birth weight, and obesity. These associations could result from changes in insulin secretion. We have analyzed glucose-stimulated insulin secretion using hyperglycemic clamps in carriers of a CA repeat in the *IGF-I* promoter and an *Apa*I polymorphism in the *IGF-II* gene. Normal and impaired glucose-tolerant subjects (n = 237) were independently recruited from three different populations in the Netherlands and Germany to allow independent replication of associations. Both first- and second-phase insulin secretion were not significantly different between the various *IGF-I* or *IGF-II* genotypes. Remarkably, noncarriers of the *IGF-I* CA repeat allele had both a reduced insulin sensitivity index (ISI) and disposition index (DI), suggesting an altered balance between insulin secretion and insulin action. Other diabetes-related parameters were not significantly different for both the *IGF-I* and *IGF-II* gene variant. We conclude that gene variants in the *IGF-I* and *IGF-II* genes are not associated with detectable variations in glucose-stimulated insulin secretion in these three independent populations. Further studies are needed to examine the exact contributions of the *IGF-I* CA repeat alleles to variations in ISI and DI. *Diabetes* 53 (Suppl. 1):S26–S30, 2004

IGFs are regulators of processes like growth and metabolism (1–3). IGF-I and IGF-II also contribute to pancreatic β-cell growth and development by regulating β-cell replication, renewal, and apoptosis (4,5). Deregulation of the balance between β-cell renewal and apoptosis due to alterations in IGF levels is potentially of great importance in the development of glucose intolerance. In addition, insulin-dependent glucose homeostasis may be affected by IGFs by sharing common steps in the signaling pathways of receptors for IGFs and insulin (6). Defects in the IGF/insulin-signaling pathways affect fetal growth and thus birth weight, which is a known risk factor for type 2 diabetes and other parts of the metabolic syndrome during life (7). Furthermore, it has been shown in animal models that ablation of the IGF-I receptor from pancreatic β-cells results in the absence of the first phase of glucose-stimulated insulin secretion and a strong reduction in second-phase insulin secretion (8,9). Together, these data make it plausible that defects at the level of IGF-I or IGF-II are associated with alterations in glucose-stimulated insulin secretion resulting in glucose intolerance.

Previously, it was shown that polymorphisms in the *IGF-I* and *IGF-II* genes are associated with features of the metabolic syndrome (10–13). A $(CA)_n$ repeat in the promoter region of the *IGF-I* gene associates with type 2 diabetes, cardiovascular disease, and reduced birth weight (10,14). Gene variants in the *IGF-II* gene are found in association studies with BMI and IGF-II levels (11,12). However, conflicting results have been reported for both gene variants (15–17). Preliminary results, based on oral glucose tolerance test (OGTT) data, have suggested a β-cell defect in carriers of gene variants in both genes (13,14; N. Vaessen, personal communication).

In this study, we analyzed glucose-stimulated insulin secretion in relation to the presence of the $(CA)_n$ repeat polymorphism in the *IGF-I* gene and the *Apa*I polymorphism in the *IGF-II* gene (10,13). As described previously, all participants underwent a hyperglycemic clamp at 10 mmol/l glucose to assess insulin secretion (18–20). Subjects with normal glucose tolerance (NGT) (n = 143) or impaired glucose tolerance (IGT) (n = 94) were recruited from three independent studies in the Netherlands and Germany (18–20). This allowed the independent replication of observations in different study cohorts, which enhances the power of our approach.

RESEARCH DESIGN AND METHODS

Subjects. Participants were selected from three independent studies in the Netherlands and Germany.

We have studied 76 subjects with NGT from Germany according the protocols of the Tübingen Family Study for Type 2 Diabetes (20). Newspaper ads and word-of-mouth proposing diabetes screens were used to recruit healthy volunteers for this study. Glucose tolerance status was assessed using

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DI, disposition index; IGT, impaired glucose tolerance; ISI, insulin sensitivity index; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; RFLP, restriction fragment length polymorphism.

OGTTs, and all subjects were GAD antibody negative and unrelated to each other. The subjects participating in the study had a mean age of 36 ± 12 years.

The Dutch NGT subjects (n = 67) were partly recruited as first-degree relatives from type 2 diabetic subjects (n = 44) (18,21). The other part of this cohort consisted of matched normoglycemic subjects. Subjects were unrelated to each other, and their mean age was 46 ± 6 years. A standard OGTT was used to define glucose tolerance status in all subjects.

Subjects with IGT (n = 94) were all of Dutch origin (19). The IGT subjects (aged 57 ± 7 years) were detected by population screening in the city of Hoorn. Subjects with a fasting glucose >5.5 mmol/l were invited for two OGTTs on separate days. Those with a mean postload glucose level between 8.6 and 11.0 mmol/l were included in the study. Also in this cohort, all subjects were unrelated to each other. The Dutch NGT and IGT groups were recruited independently in different regions of the country. Details of the study groups are described previously (18–20). Informed consent was obtained from all participants, and the appropriate local medical ethics committees approved the protocol.

Hyperglycemic clamps. Hyperglycemic clamps were performed at 10 mmol/l in all subjects. The Dutch NGT and IGT subjects underwent a 3-h clamp. In the German NGT subjects, the clamp lasted for 2 h. After an overnight fast, the subjects received an intravenous glucose bolus to acutely raise glucose levels to 10 mmol/l. Blood glucose levels were measured at the appropriate intervals to maintain a constant blood glucose during the clamp. Blood samples for insulin were drawn at 2.5-min intervals during the first 10 min of the clamp and at 10- to 20-min intervals during the remainder. Exact details of the clamping procedures in the different study groups were described previously (18–20,22). First-phase insulin secretion was defined as the sum of the insulin levels during the first 10 min of the clamp. Second-phase insulin secretion was defined as the mean of the insulin values during the last 40 min (80–120 min, NGT group, Germany) or the last 30 min (150–180 min, NGT and IGT groups, the Netherlands) of the clamp. The insulin sensitivity index (ISI) was determined by relating the glucose infusion rate to the plasma insulin concentration during the last 40 min (NGT, Germany) or 30 min of the clamp. The disposition index (DI) was calculated as the product of first-phase insulin secretion and ISI according to Bergman et al. (23).

Genotyping. Classification of the repeat length in the *IGF-I* gene was as described previously (10); carriers of the normal 192-bp allele had a repeat length of 19 CA repeat units. All other repeat lengths were classified as noncarrier (10). Genotypes of the *Apa*I polymorphism in the *IGF-II* gene were determined by a PCR–restriction fragment length polymorphism (RFLP)-based method or direct sequencing as described previously (13). The most prevalent GG genotype was used as a reference.

Statistics. All data are presented as means ± SE or median with interquartile range. ANOVA or Mann-Whitney U test was used for general comparisons between the different genotypes. Variables were log-transformed before analysis if necessary. Adjustments for age, sex, BMI, and study center in the pooled analyses were done in separate general linear regression analyses for all parameters. A priori power calculations showed that the design used in this study would allow the detection of a difference in first or second phase of insulin secretion between 15 and 30% with 90% power ($P \leq 0.05$). Results were regarded significant at $P \leq 0.05$. Statistical analysis was performed with the SPSS version 10.0 software (SPSS, Chicago).

RESULTS

We used three different cohorts in our studies. One of the cohorts consisted of randomly recruited NGT subjects from Germany. The second cohort of NGT subjects was in part recruited as first-degree relatives of type 2 diabetic individuals. The third cohort consisted of subjects with IGT (18–20). The last two cohorts were independently selected from two different regions in the Netherlands and had both an increased risk for type 2 diabetes and evidence for β-cell dysfunction (22). As can be expected, the IGT group had the lowest insulin secretion levels, ISIs, and DIs compared with the other two NGT groups (21). Classification of the repeat length in the *IGF-I* gene was as described previously (10); carriers of the normal 192-bp allele had a repeat length of 19 CA repeat units. All other repeat lengths were classified as noncarrier (10). Genotypes of the *Apa*I polymorphism in the *IGF-II* gene were determined by a PCR-RFLP–based method as described previously (13). Genotype frequencies for the *IGF-I* and *IGF-II* variants were comparable between the different cohorts and are comparable to those reported in other Caucasian populations (Tables 1 and 2) (10–12,15). All genotype distributions were in the Hardy-Weinberg equilibrium (data not shown).

IGF-I promoter gene variant. We have investigated whether the *IGF-I* gene variant is associated with insulin secretion in two independent populations from the Netherlands. Glucose-stimulated insulin secretion during the hyperglycemic clamp was not significantly different between the different genotypes, both under a recessive or dominant model (Table 1). However, we do see a reduced ISI and DI in IGT noncarriers of the 192-bp allele (P for trend <0.05, Table 1). Combined analysis of the Dutch NGT and IGT groups resulted in a significant association of the polymorphism with clamp-derived insulin sensitivity (ISI) (P = 0.01 for 192 bp/192 bp + non-192 bp/192 bp vs. non-192 bp/non-192 bp, with adjustment for age, sex, BMI, and study center). Furthermore, we noted a decreased DI in noncarriers of the 192-bp allele (P = 0.03 for trend, with adjustment for age, sex, BMI, and study center). Other diabetes-related parameters such as glucose and insulin levels were not significantly different between the different *IGF-I* genotypes (Table 1). There was no evidence for an interaction with BMI (data not shown). Data about the *IGF-I* polymorphism in the German NGT cohort are not available.

IGF-II gene variant. Because of the low number of AA carriers, we pooled the AA and AG genotypes of the *IGF-II* gene variant in most of our analyses (Table 3). Separate testing of the AA carriers did not essentially change our findings. Glucose and insulin levels during OGTTs were not significantly different between the two genotype groups in either of the study populations (data not shown). Also, the first phase of the glucose-stimulated insulin secretion, as measured by hyperglycemic clamps, was not different between the genotypes (adjusted for age, sex, and BMI) (Table 3). We did see, however, a decreased second-phase insulin secretion in the NGT group from the Netherlands (P = 0.04 for GG vs. AA + AG, adjusted for age, sex, and BMI, Table 3). This was, however, not replicated in the two other cohorts (Table 3). If BMI is a mediating factor in the causal chain of the gene variant leading to alterations in glucose metabolism, adjustment for BMI would be an over-adjustment. However, when we repeated the tests without BMI in the model, the results did not change.

Insulin sensitivity, as assessed with the hyperglycemic clamps, was not significantly different between the genotypes in all our cohorts (Table 3). Furthermore, there was no evidence for a gene-environment interaction with factors such as BMI and triglycerides (data not shown). However, trends toward lower body weight and BMIs were observed in carriers of the AA genotype without reaching statistical significance in any of the populations (Table 2). A pooled analysis of all three study cohorts did not change our findings (P > 0.1, data not shown). Furthermore, we did not observe significant changes in fasting glucose and (pro)insulin levels, lipid profiles, and other diabetes- and/or obesity-related parameters (Table 2, data not shown).

TABLE 1
Clinical characteristics according to the *IGF-I* $(CA)_n$ repeat genotype

	NGT				IGT			
	192/192 bp	192/non-192 bp	non-192/non-192 bp	*P*	192/192 bp	192/non-192 bp	non-192/non-192 bp	*P*
n (M/F)	31 (8/23)	26 (7/19)	6 (0/6)		43 (24/19)	43 (18/25)	8 (3/5)	
Age (years)	46 ± 1	45 ± 1	50 ± 4	0.18	58 ± 1	57 ± 1	54 ± 3	0.32
BMI (kg/m^2)	25.7 ± 0.7	25.8 ± 0.6	27.0 ± 2.5	0.80	28.2 ± 0.5	28.5 ± 0.7	27.9 ± 1.1	0.85
Fasting glucose (mmol/l)	4.6 ± 0.1	4.5 ± 0.1	4.5 ± 0.1	0.58	6.6 ± 0.8	6.6 ± 0.1	6.5 ± 0.2	0.93
Fasting insulin (pmol/l)	30 (24–48)	30 (24–42)	36 (24–54)	0.41	72 (57–96)	67 (49–102)	73 (63–97)	0.96
First-phase insulin secretion (pmol/l)	918 (648–1,332)	768 (606–1,041)	1044 (818–1,310)	0.24	584 (365–909)	541 (318–831)	749 (336–790)	0.40
Second-phase insulin secretion (pmol/l)	274 (190–522)	313 (251–449)	321 (280–819)	0.53	216 (150–362)	198 (129–401)	345 (299–425)	0.54
ISI (μmol · kg^{-1} · min^{-1} · pmol^{-1} · l^{-1})*	0.17 (0.11–0.28)	0.17 (0.13–0.22)	0.10 (0.07–0.18)	0.39	0.13 (0.08–0.18)	0.10 (0.06–0.16)	0.09 (0.04–0.13)	0.02
DI (μmol · kg^{-1} · min^{-1})†	160 (105–200)	128 (106–166)	104 (67–159)	0.41	93 (42–155)	68 (41–97)	49 (29–75)	0.04

Data are means ± SE or medians (interquartile range). *P* values for trend were obtained after ANOVA or linear regression analysis with adjustment for age, sex, and BMI. *$P = 0.01$ for a combined analysis of the NGT and IGT groups (non-192/non-192 vs. non-192/192 + 192/192 bp) adjusted for age, sex, study center, and BMI. †$P = 0.03$ for trend in a combined analysis of the NGT and IGT groups, adjusted for age, sex, study center, and BMI.

TABLE 2
Clinical characteristics in NGT and IGT subjects according to the *IGF-II Apa1* genotype

	NGT (Germany)				NGT (the Netherlands)				IGT (the Netherlands)			
	AA	AG	GG	*P*	AA	AG	GG	*P*	AA	AG	GG	*P*
n	3	32	41		4	31	32		6	35	53	
Age (years)	47.3 ± 9.7	33.3 ± 1.7	37.1 ± 2.1	0.12	43.5 ± 2.5	46.0 ± 1.3	45.8 ± 1.1	0.77	55.7 ± 3.9	58.1 ± 1.2	56.5 ± 1.0	0.55
Sex (M/F)	1/2	15/17	19/22	0.91	4/0	8/23	8/24	0.52	4/2	18/17	23/29	0.53
Body weight (kg)	60.7 ± 4.0	73.6 ± 2.5	75.0 ± 3.1	0.38	70.9 ± 9.5	77.3 ± 2.4	74.4 ± 2.1	0.51	81.5 ± 3.4	83.6 ± 2.6	81.9 ± 1.8	0.84
BMI (kg/m^2)	21.2 ± 0.9	24.4 ± 0.8	25.4 ± 1.0	0.38	25.4 ± 2.0	26.3 ± 0.8	25.2 ± 0.6	0.48	26.8 ± 0.8	28.8 ± 0.7	28.4 ± 0.5	0.50
Waist-to-hip ratio	0.77 ± 0.05	0.84 ± 0.01	0.86 ± 0.01	0.19	0.81 ± 0.01	0.82 ± 0.01	0.80 ± 0.05	0.72	0.94 ± 0.04	0.93 ± 0.02	0.93 ± 0.01	0.99
Triglycerides (pmol/l)	NA	NA	NA		1.17 ± 0.40	1.12 ± 0.08	1.06 ± 0.10	0.86	1.98 ± 0.20	2.22 ± 0.22	2.21 ± 0.19	0.92

Data are means ± SE. Differences between the genotypes were tested by ANOVA. A pooled analysis of all three groups did not yield significant differences (with correction for age and sex). NA, not available.

TABLE 3
Hyperglycemic clamp results in NGT and IGT subjects according to *IGF-II Apa1* genotype

	NGT (Germany)			NGT (the Netherlands)			IGT (the Netherlands)		
	AA + AG	GG	*P*	AA + AG	GG	*P*	AA + AG	GG	*P*
n (%)	35 (0.46)	41 (0.54)	—	35 (0.52)	32 (0.48)		41 (0.44)	53 (0.56)	—
Fasting plasma glucose (mmol/l)	4.8 ± 0.1	4.9 ± 0.2	0.68	4.6 ± 0.1	4.5 ± 0.1	0.45	6.6 ± 0.1	6.6 ± 0.1	0.75
Fasting plasma insulin (pmol/l)	32 (26–54)	37 (26–53)	0.13	32 (24–46)	30 (24–40)	1.00	66 (50–88)	69 (45–107)	0.86
First-phase insulin secretion (pmol/l)	801 (541–1,112)	663 (497–1,107)	0.80	924 (726–1,326)	828 (530–1,077)	0.16	560 (333–881)	570 (361–878)	0.76
Second-phase insulin secretion (pmol/l)*	224 (144–307)	224 (149–310)	0.58	340 (274–522)	277 (191–432)	0.04	220 (133–373)	205 (144–384)	0.55
ISI (μmol · kg^{-1} · min^{-1} · pmol^{-1} · l^{-1})	0.16 (0.09–0.27)	0.16 (0.09–0.22)	0.76	0.14 (0.09–0.23)	0.18 (0.13–0.26)	0.08	0.10 (0.07–0.16)	0.12 (0.06–0.17)	0.92
DI (μmol · kg^{-1} · min^{-1})	110 (70–156)	115 (69–176)	0.37	136 (77–183)	156 (110–199)	0.95	58 (38–93)	82 (42–120)	0.62

Data are *n* (%), means ± SE, or medians (interquartile range). *Second-phase insulin secretion was calculated as the average insulin level from 80 to 120 min in the German NGT subjects and the average insulin level from 150 to 180 min in both the Dutch NGT and IGT subjects. Differences between the genotypes were tested by ANOVA and/or linear regression analysis. *P* values were obtained after adjustment for age, sex, and BMI.

DISCUSSION

It has been shown previously that IGF-I and IGF-II levels are determinants of fetal growth and birth weight in both humans and animals. Reduced birth weight is a known risk factor for the development of diabetes and other features of the metabolic syndrome (7). Genetically determined low levels of IGFs may in part explain these observations. Previously it was shown that both the *IGF-I* and *IGF-II* gene variants examined in this study are associated with reduced levels of the respective growth factors (10,11). IGFs are also important factors for the delicate balance between cell survival and cell death in the pancreatic β-cell (4). A disturbed balance between β-cell replication and apoptosis might result in an altered setting of the β-cell, eventually resulting in β-cell failure. IGF-I and IGF-II bind to specific receptors on the pancreatic β-cell. One of these receptors is the IGF-I receptor (8). Studies using β-cell–specific IGF-I receptor knockouts have shown that these animals have an almost absent first and second phase of glucose-stimulated insulin secretion (8,9). Because β-cell mass is unaffected, this might be caused by altered glucose-sensing capacities of the β-cells lacking the IGF-I receptor (8). Together, this has led us to speculate that genetic defects in both IGF-I and IGF-II alter glucose-stimulated insulin secretion in humans. Preliminary data suggested a secretion defect in both carriers of the *IGF-I* and *IGF-II* gene variants (13,14).

In this study, we have examined glucose-stimulated insulin secretion in relation to these gene variants. From our data, we conclude that the two gene variants in the IGF genes are not associated with the magnitude of glucose-stimulated insulin secretion during hyperglycemic clamps in independent Dutch and German subjects. This suggests that the mild variations in IGF levels associated with the gene variants in *IGF-I* and *IGF-II* do not affect glucose-stimulated insulin secretion. This is further corroborated by the fact that IGF-I levels are not associated with glucose-stimulated insulin secretion (24). We have examined three cohorts with either NGT or IGT originating from three independent populations in the Netherlands and Germany. A limited part of our populations consisted of first-degree relatives of type 2 diabetic individuals and subjects with IGT (18,19). Both groups have an increased risk of developing type 2 diabetes (22). One of the early defects in these at-risk individuals is a reduced first-phase insulin secretion (22). If mutations in the IGF genes are associated with altered glucose-stimulated insulin secretion, we expect this should be first manifest in these at-risk individuals. With respect to changes in (first-phase) insulin secretion, we did not observe any association. We cannot, however, exclude the possibility that changes in insulin secretion only become manifest after stressing glucose homeostasis, for instance during overfeeding. Previously, it has been shown that carriers of the *IGF-II* gene variant have an altered insulin response during an OGTT after a 100-day period of overfeeding (13).

We observed a reduced ISI and DI associated with the CA repeat polymorphism in the *IGF-I* gene, suggesting an altered balance between insulin sensitivity and insulin secretion. Previously, it has been shown that the ISI as measured with the hyperglycemic clamp technique correlates very well with the values obtained by the "gold

standard" hyperinsulinemic-euglycemic clamp (25). It has also been shown that a hyperbolic relationship exists between insulin secretion and insulin sensitivity. This hyperbolic relationship can be mathematically described by the multiplication of indexes for (first-phase) insulin secretion and insulin sensitivity, which is referred to as the disposition index (23). Subjects with type 2 diabetes have a lower DI than normoglycemic subjects. A low DI indicates an inability of the β-cell to adapt adequately to changes in insulin sensitivity. It is conceivable that, under circumstances of deterioration of insulin action, as occurs in obesity, this situation will lead to (further) deterioration of glucose homeostasis and may consequently lead to type 2 diabetes. Whether this is the case in noncarriers of the normal CA repeat allele of the *IGF-I* promoter gene is so far not known.

The exact mechanism by which IGF-I affects insulin sensitivity is unclear but might involve shared components of the IGF-I/insulin-signaling pathway (6). This phenomenon has been observed in mice lacking IGF-I expression in the liver. These mice show muscle insulin resistance most likely due to defects at the level of the insulin receptor (26). Furthermore, it is known that IGF-I levels affect insulin sensitivity in humans as well (3). Further studies are necessary to examine the relationship between the CA repeat in the *IGF-I* promoter and insulin resistance and reduced DI in detail.

We conclude that gene variants in the *IGF-I* and *IGF-II* genes are not associated with detectable alterations in glucose-stimulated insulin secretion in three independent study populations from the Netherlands and Germany. However, our findings of a reduced ISI and a reduced DI in noncarriers of the normal CA repeat allele in the *IGF-I* promoter may point to a disturbed adaptation of β-cells to insulin action and warrants further study, especially in obese (insulin-resistant) subjects.

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