Brief Genetics Report

A Putative Functional Polymorphism in the *IGF-I* Gene

Association Studies With Type 2 Diabetes, Adult Height, Glucose Tolerance, and Fetal Growth in U.K. Populations

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IGF-I has a critical role in growth and metabolism. A microsatellite polymorphism 1 kb upstream to the *IGF-I* gene has recently been associated with several adult phenotypes. In a large Dutch cohort, the absence of the commonest allele (Z) was associated with reduced serum IGF-I levels, reduced height, and an increased risk of type 2 diabetes and myocardial infarction. This result has not been replicated, and the role of this polymorphism in these traits in U.K. subjects is not known. We sought further evidence for the involvement of this variant in type 2 diabetes using a case-control study and IGF-I and diabetes-related traits in a population cohort of 640 U.K. individuals aged 25 years. Absence of the common allele was not associated with type 2 diabetes (odds ratio 0.70, 95% CI 0.47–1.04 for X/X versus Z/Z genotype, χ^2 test for trend across genotypes, $P = 0.018$). In the population cohort, the common allele (Z) was associated with decreased IGF-I levels ($P = 0.01$), contrary to the Dutch study, but not with adult height ($P = 0.23$), glucose tolerance ($P = 0.84$), oral glucose tolerance test–derived values of β-cell function ($P = 0.90$), or insulin resistance ($P = 0.66$). There was no association with measures of fetal growth, including birth weight ($P = 0.17$). Our results do not support the previous associations and suggest that the promoter microsatellite is unlikely to be functionally important. *Diabetes* 51:2313–2316, 2002

Recently, Vaessen et al. (1), in a study of 900 Dutch individuals from Rotterdam, the Netherlands, described genetic variation upstream of the *IGF-I* gene that was associated with altered growth and glucose tolerance. A microsatellite polymorphism 1 kb upstream to the *IGF-I* gene was associated with several adult phenotypes. The absence of the commonest 192-bp allele was associated with reduced serum IGF-I levels ($P = 0.003$), reduced height ($P = 0.004$), and an increased risk of type 2 diabetes (odds ratio [OR] 1.7, 95% CI 1.1–2.7). A previous study of 82 Danish subjects did not reveal any common (frequency >0.02) coding variation in the *IGF-I* gene (2). The *IGF-I* gene lies on chromosome 12q21 between 106 and 113 cM, a region that is close to a region (12q24) linked to type 2 diabetes in Scandinavian subjects (3,4), but probably not close enough to contribute to evidence for linkage.

Serum IGF-I levels are strongly associated with both fetal and postnatal growth (5). This together with the data from Vaessen et al. (1) make variation at the *IGF-I* gene an ideal candidate for influencing growth and diabetes risk. The role of the *IGF-I* promoter polymorphism in U.K. populations is not known. We used cohorts of type 2 diabetic and control subjects (both from Devon, U.K.) and a cohort of young adults from the Barry-Caerphilly Growth (BCG) study (see below) to assess the role of the *IGF-I* variant in determining type 2 diabetes risk, IGF-I levels, intermediate traits related to type 2 diabetes, and measures of fetal and postnatal growth.

Table 1 gives allele and genotype frequencies in our cohorts. These were all in Hardy-Weinberg equilibrium. Tables 2 and 3 give descriptive data on the cohorts used. Table 4 shows data from the BCG cohort on a variety of outcome measures according to whether subjects carried 0, 1, or 2 common alleles.

Initially, we sought replication of the association between IGF-I genotypes and type 2 diabetes risk. In contrast to the Dutch study (1), the absence of the common allele Z was associated with reduced diabetes risk (OR for absence of Z allele 0.79, 95% CI 0.63–0.98, $P = 0.03$; trend across the three genotypes, $P = 0.018$).

We next sought evidence for an association between the *IGF-I* promoter polymorphism and IGF-I levels in the BCG cohort of 640 individuals. Subjects with the Z allele had lower IGF-I levels before and after correction for IGFBP-3 levels (P value test for trend = 0.01) (Table 4), although this did not show a dose-response effect, being driven by lower values in the Z/Z genotype group. In the same data set, there were no significant effects of genotype on adult height (Table 4), weight, or BMI (data not shown).

We hypothesized that if this variant influences susceptibility to type 2 diabetes (or is in linkage disequilibrium

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BCG, Barry-Caerphilly Growth; HOMA-S, homeostasis model assessment for insulin sensitivity; LD, linkage disequilibrium; OGTT, oral glucose tolerance test; OR, odds ratio.

TABLE 1
Allele distribution of the IGF-I promoter polymorphism in U.K. and Dutch populations

Allele	Genotype	Devon, U.K., diabetic subjects (n = 348)	Devon, U.K., control subjects (n = 365)	BCG population cohort (n = 640)	Dutch diabetic subjects (n = 220)*	Dutch control subjects (n = 596)*
Z+8		1 (0.1)	—	—		
Z+6		12 (1.7)	20 (2.7)	28 (2.2)		
Z+4		31 (4.4)	53 (7.2)	93 (7.3)		
Z+2		136 (19.5)	142 (19.5)	240 (18.8)		
Z		470 (67.5)	453 (62.1)	805 (62.9)		
Z−2		33 (4.7)	44 (6.0)	77 (6.0)		
Z−4		12 (1.7)	15 (2.1)	29 (2.3)		
Z−6		—	2 (0.3)	1 (0.1)		
Z−16		1 (0.1)	—	7 (0.5)		
	Z/Z	161 (46.4)	136 (37.3)	254 (39.7)	83 (37.7)	277 (46.5)
	Z/X	148 (42.0)	181 (49.6)	297 (46.4)	102 (46.4)	248 (41.6)
	X/X	39 (11.4)	48 (13.2)	89 (13.9)	35 (15.9)	71 (11.9)
				P = 0.018†		

Data are n of alleles and genotype (%). Z = common allele, 192 bp in Vaessen et al. study (1); X = all other alleles. *From Vaessen et al.; †two-tailed P value for Z allele trend across genotypes when comparing all U.K. control subjects (n = 1005) with U.K. diabetic subjects (n = 348).

[LD] with a nearby disease variant), it would also be associated with one or more diabetes-related intermediate traits. We did not see any differences (at $P < 0.05$) between any of the following traits and *IGF-I* genotype in the BCG cohort of 640 individuals: glucose tolerance as measured by fasting and 2-h OGTT levels, β-cell function as measured by insulinogenic index, and insulin sensitivity as measured by fasting, 2-h insulin, or homeostasis model assessment for insulin sensitivity (HOMA-S) (Table 4).

The crucial role of IGF-I in growth led us to seek evidence for association between the promoter polymorphism and measures of fetal and postnatal growth. No association (at $P < 0.05$) was seen with birth weight, head circumference, or length at birth (Table 4). To address the potential confounding factor of maternal influence on fetal growth, we performed the same subanalysis as described by Dunger et al. (6), with subjects split into those that moved through ≥0.67 growth centile SDs between birth and 2 years (changers) and those who did not (nonchangers). However, we did not observe any increased evidence for associations between *IGF-I* genotype and any birth traits in either the nonchangers or changers (data not shown). When examining adult phenotypes, the only nominally significant observations were seen in the changers, in whom Z/Z homozygotes had increased BMI and weight (P = 0.028 and 0.026, respectively). These were not significant (at $P < 0.05$) when the number of tests performed were taken into account.

To explore the possibility that different patterns of LD may exist between the Dutch and U.K. populations, and therefore explain the discrepant results, we further analyzed our data for associations using the eight genotypes with >10 observations versus the homozygous Z genotype. None of the results remained significant (at $P < 0.05$) after a Bonferonni correction for multiple comparisons. Full results are available from the authors on request.

In this study of U.K. subjects, we assessed the role of the *IGF-I* gene promoter polymorphism in type 2 diabetes as well as several traits related to type 2 diabetes, including growth. The known role of IGF-I in growth and the association between fetal growth and type 2 diabetes (7) makes *IGF-I* an excellent candidate gene for these phenotypes. The association we observed between the Z allele of the polymorphism and type 2 diabetes was opposite to that found in the Dutch population. We also observed significantly reduced serum IGF-I levels in subjects with two copies of the common allele of the *IGF-I* promoter polymorphism (Table 3), also opposite that found in the Dutch study. Our IGF-I result is in keeping with a previous study looking at this polymorphism in relation to bone mineral density (8). A further study demonstrated reduced IGF-I levels in women using oral contraceptives with at least one copy of the Z allele (P = 0.04), but this effect was not present when oral contraceptive use was excluded from the analysis (9). Our results, unlike those of Vaessen et al. (1), indicate that the polymorphism itself is not functional. If both results represent a true association, this would mean there was greatly differing LD between the polymor-

TABLE 2
Details of subjects in the case-control study

	Diabetic subjects (n = 348)		
	Sib-pair probands	Young-onset type 2 diabetes	Control subjects
N (M/F)	155 (88/67)	193 (110/83)	363 (181/182)
Age (years)*	56 (50–63)	40.6 (36.5–44.5)	32 (29–35)
BMI (kg/m^2)	27.1 (24.5–30.7)	30.3 (26.7–34.2)	26.5 (24.1–21.3)†
Treatment (% diet/OHA/insulin)	16/63/20	12/37/51	—

Data are median (interquartile range), unless otherwise indicated. *Age at diagnosis in diabetic subjects and age at study in control subjects. †Male subjects only; weight of female subjects only available when pregnant. OHA, oral hypoglycemic agent.

TABLE 3
Basic descriptive data for the BCG population cohort by sex

Variable	Male patients	Female patients
n	342	298
Age (years)	25.0	25.0
Adult height (cm)	177	164
IGF-I (ng/ml)*	139.2	144.0
IGFBP-3 (μg/ml)†	6.33	6.49
IGF-I/IGFBP-3 ratio*	22.1	21.9
BMI (kg/m^2)	25.1	25.2
Fasting glucose (mmol/l)†	4.71	4.44
2-h glucose (mmol/l)†	4.85	5.31
Fasting insulin (pmol/l)†	39.3	42.5
2-h insulin (pmol/l)†	133.0	225.9
HOMA-S†	679	944
Insulinogenic index†	112	136
Birth weight (kg)	3.45	3.31
Birth length (cm)	52.4	51.3
Birth head circumference (cm)	36.4	35.4

*Based on square root transformation; †geometric mean.

phism and an unknown functional variant. This is unexpected in two closely related Northern European populations. Additional studies are needed to further assess any association between this polymorphism and type 2 diabetes risk and IGF-I levels.

We did not find any associated phenotype to support the evidence that IGF-I levels were influenced by the polymorphism (or a variant in LD with the promoter microsatellite). In particular, in contrast to the Dutch study, we did not find any associations ($P = 0.23$) between this polymorphism and adult height. The absence of any associations with fasting ($P = 0.84$) or 2-h oral glucose tolerance test (OGTT) glucose ($P = 0.84$), insulinogenic index ($P = 0.90$), or fasting insulin ($P = 0.34$) in our BCG population cohort does not provide any additional support for a role of the *IGF-I* polymorphism in type 2 diabetes susceptibility. However, it is possible that these subjects were too young for us to observe any alterations in glucose tolerance.

There are a number of possible reasons why we have not replicated the findings of Vaessen et al. (1): *1*) chance—given the moderate *P* values obtained in the two studies false-positive results are possible. A recent review highlighted the importance of building up evidence over many studies for or against the role of genetic variation in common traits (10). *2*) Differences between subjects in the two studies—our diabetic subjects were diagnosed 20 years younger, on average, than those in the Dutch study. Also, the BCG subjects were born in Wales in the early 1970s, whereas the Rotterdam subjects who were born in the 1930s; therefore, dietary and other environmental differences during childhood may explain the differences in results. *3*) Different patterns of LD between the two populations may partly explain the discrepancies.

In conclusion, using well-characterized cohorts of U.K. subjects, we have not replicated the associations seen in the Rotterdam study between variation at the *IGF-I* promoter and type 2 diabetes susceptibility, IGF-I levels, and growth. Instead, we observed weak associations with type 2 diabetes and decreased IGF-I levels, results contrary to the Dutch study. We did not obtain any support for these findings because there was no association with intermediate traits such as β-cell function, insulin sensitivity, fetal growth, or postnatal growth. Our results therefore do not suggest that this promoter polymorphism is a functional variant. Further large studies are needed to establish if genetic variation in the *IGF-I* gene is associated with altered IGF-I levels or type 2 diabetes.

RESEARCH DESIGN AND METHODS

Subjects

Case-control study. We used 348 unrelated type 2 diabetic subjects living in Devon, U.K. These subjects were selected to be enriched for a family history of type 2 diabetes, either by young age of diagnosis ($n = 193$ diagnosed <45 years

TABLE 4
Adjusted mean values of adult outcome measures by *IGF-I* promoter genotype

	Genotype					*P* (test for trend)
	X/X	X/Z	*P*	Z/Z	*P*	
Adult IGF-related outcomes						
Adult height (cm)	177.07 (175.72–178.42)	177.11 (176.27–177.95)	0.95	177.78 (176.89–178.67)	0.35	0.23
IGF-I levels (ng/ml)	142.80 (133.40–152.52)	143.52 (137.59–149.57)	0.89	133.17 (127.24–139.48)	0.07	0.01
IGFBP-3 levels (μg/ml)	6.42 (6.05–6.82)	6.30 (6.05–6.49)	0.52	6.36 (6.11–6.62)	0.87	0.91
IGF-I/IGFBP-3 ratio	22.56 (20.98–24.21)	22.85 (21.90–23.91)	0.714	21.07 (20.07–22.09)	0.10	0.02
Adult OGTT measures						
Fasting glucose (mmol/l)	4.76 (4.66–4.81)	4.71 (4.66–4.76)	0.64	4.76 (4.66–4.81)	1.00	0.84
2-h glucose (mmol/l)	4.85 (4.62–5.16)	4.85 (4.71–5.05)	0.93	4.85 (4.66–5.00)	0.91	0.84
Fasting insulin (pmol/l)	40.0 (35.9–44.7)	38.1 (35.5–40.9)	0.43	40.9 (38.1–44.3)	0.66	0.34
2-h insulin (pmol/l)	138 (118–162)	132 (119–145)	0.57	133 (119–147)	0.64	0.75
HOMA-S	679 (602–773)	665 (602–713)	0.7	685 (633–750)	0.84	0.66
Insulinogenic index	117 (98–141)	109 (98–123)	0.49	116 (101–130)	0.90	0.90
Fetal outcome measures						
Birth weight (kg)	3.49 (3.385–3.6)	3.45 (3.38–3.519)	0.49	3.42 (3.35–3.493)	0.24	0.22
Z-score birth weight	0.111 (−0.107–0.329)	0.008 (−0.129–0.144)	0.39	−0.059 (−0.207–0.089)	0.17	0.17
Z-score birth length	0.056 (−0.162–0.275)	−0.016 (−0.153–0.121)	0.55	0.011 (−0.137–0.159)	0.72	0.86
Z-score head circumference	0.098 (−0.12–0.317)	−0.045 (−0.183–0.092)	0.24	0.011 (−0.137–0.159)	0.49	0.74

Data are means (95% CI). All variables adjusted to male sex and average age. IGFBP-3, fasting and 2-h glucose and insulin, HOMA index, and insulinogenic index are back transformed from natural log variables. IGF-I and IGF-I/IGFBP-3 ratio have been back transformed from square root transformation. Z indicates common allele (192 bp in Vaessen et al. [1]) and X all other alleles. *P* values are for genotype group versus X/X genotype.

of age, young-onset type 2 diabetes) or because they had an affected sibling (n = 155 sibling-pair probands). All affected subjects were GAD autoantibody negative.

Control subjects consisted of parents from a consecutive birth cohort with normal (<5.5 mmol/l) fasting glucose and normal (<6.0%) HbA_{1c} levels (Diabetes Control and Complications Trial corrected). All subjects were of white U.K. origin and were residents of the county of Devon, U.K.

Population cohort: the BCG cohort. The original study was a randomized-controlled trial undertaken between 1972 and 1974 in Barry and Caerphilly, South Wales (11). All eligible pregnant mothers (1,251 participants, 97% response rate) were approached for recruitment and randomized to either a "supplemented" or control group. Women in the supplemented group were provided with milk tokens throughout pregnancy and until their child reached 5 years of age (951 participants, 76%), when the original study terminated. Each token was equivalent to the price of half a pint of milk and was accepted by the local milk deliverymen.

Birth weight was obtained from hospital records, and subjects were weighed on a portable beam balance. Crown-heel length was measured on a specially developed and modified infant stadiometer (12). Birth length was measured at 10 days to give time for any caput to resolve. Height, at 5 years of age, was measured on a stadiometer with stretching. The maximum occipito-frontal measurement was taken with a disposable paper tape measure for head circumference. Trained study nurses, who were monitored throughout the study, made all the measurements, except birth weight.

Between 1997 and 1999, all the original children who had completed the 5-year follow-up were traced through their parents or by invitation from the relevant health authority after flagging the subjects through the National Health Service Central Register. Subjects were invited to complete a questionnaire and attend a screening clinic (679 participants, 71% response rate). At the clinic, subjects had their height (standing and sitting) and weight typically measured twice by a single observer. The average of these two values was taken, unless there was only one reading. Two subjects with known insulin-dependent diabetes were excluded from an OGTT. Fasting blood samples were obtained before the administration of a 75-g oral glucose challenge, consumed by the subject at a steady rate over 5 min (13). Blood samples were also taken at 30 and 120 min.

Glucose was measured using a standard assay and insulin levels by an enzyme-linked immunosorbent assay (ELISA K6219; DAKO). IGF-I and IGFBP-3 were assayed by radioimmunoassays as previously described (14). IGF-I was assayed after an acid-acetone extraction to remove binding proteins, followed by the addition of excess IGF-II to block any remaining binding sites. Correcting IGF-I levels for IGFBP-3 levels using the IGF-I/IGFBP-3 ratio gives an index of biologically available hormones. HOMA-S was calculated using the following equation: (20 × fasting insulin)/(3.5 × fasting glucose). Insulinogenic index was calculated from OGTT data as (30-min insulin − fasting insulin)/(30-min glucose − fasting glucose).

Genotyping. The CA repeat microsatellite polymorphism, situated ~1,000 bp 5′ of the transcription start site of *IGF-I*, was amplified by PCR using the following primers: forward: 5′-ACCACTCTGGGAGAAGGGTA and reverse: 5′-GTTTGCTAGCCAGCTGGTGTTATT, with 0.25 units Amplitaq Gold, 0.25 mmol/l dNTPs, 0.15 mmol/l $MgCl_2$, and ~40 ng DNA. A 5′ GTTT "PIGtail" was added to the reverse primer to reduce the occurrence of nontemplated A addition. The forward primer was 5′ labeled with the FAM fluorochrome. Products were separated on a 4.25% sequencing gel using an automated 377 sequencer and analyzed using GENESCAN and GENOTYPER software (ABI Applied Biosystems). A total of 84 randomly selected samples (13%) were retyped to check for inconsistencies. All genotypes were scored separately by two individuals, and ambiguous genotypes were retyped. There was no deviation from the expected Hardy-Weinberg equilibrium (P = 0.99).

Statistics. Hardy-Weinberg equilibrium at the *IGF-I* microsatellite was assessed by χ^2 comparisons of observed genotype frequencies, with expected genotype frequencies inferred from observed allele frequencies.

Standard contingency tables were used to calculate ORs, and χ^2 analyses were performed to generate P values. Epi-info 2000 (Version 1.1) was used to estimate the power of our case-control study. By using both the BCG and Devon control populations as a combined control population (the two cohorts had very similar allele frequencies), we had 93% power to detect the OR of 1.7 estimated from the Dutch study and 80% power to detect an OR of 1.53 in the same direction as the Dutch study (at $P \leq 0.05$).

Anthropometric data were transformed from their raw values to a sex-specific Z-score distribution. This was internally derived using all subjects for whom there was genotype data. For birth dimensions, all babies born before 36 and after 44 weeks were excluded, and standardization was undertaken within sex and stratified by gestational week. For all the other anthropometric measures, sex-specific Z-score values were derived after adjusting for the age of the subject at the time of measurement. IGF-I and IGF-I/IGFBP-3 ratio were transformed using a square root transformation. IGFBP-3, fasting and 2-h glucose and insulin, HOMA index, and insulinogenic index were log transformed.

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