Brief Genetics Report

Polymorphisms in the Insulin-Degrading Enzyme Gene Are Associated With Type 2 Diabetes in Men From the NHLBI Framingham Heart Study

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Linkage studies have mapped a susceptibility gene for type 2 diabetes to the long arm of chromosome 10, where we have previously identified a quantitative trait locus that affects fasting blood glucose within the Framingham Heart Study cohort. One candidate gene in this region is the insulin-degrading enzyme (IDE), which, in the GK rat model, has been associated with nonobese type 2 diabetes. Single nucleotide polymorphisms (SNPs) were used to map a haplotype block in the 3′ end of IDE, which revealed association with HbA_{1c}, fasting plasma glucose (FPG), and mean fasting plasma glucose (mFPG) measured over 20 years. The strongest associations were found in a sample of unrelated men. The lowest trait values were associated with a haplotype (TT, f~0.32) containing the minor allele of rs2209772 and the major allele of the rs1887922 SNP (FPG $P < 0.001$, mFPG $P < 0.003$, HbA_{1c} $P < 0.025$). Another haplotype (CC, f~0.16) was associated with elevated HbA_{1c} ($P < 0.002$) and type 2 diabetes ($P < 0.001$, odds ratio 1.96, 95% CI 1.28–3.00). The evidence presented supports the possibility that IDE is a susceptibility gene for diabetes in populations of European descent. ***Diabetes*** **52:1562–1567, 2003**

Previous studies have identified linkage to chromosome 10q23-q25 for fasting plasma glucose (FPG) (1), mean fasting plasma glucose (mFPG) levels measured over 20 years (1), HbA_{1c} (1), type 2 diabetes (2), and the ratio of fasting insulin to fasting glucose (3). The apparent replication of linkage in different studies using populations of mixed European descent made this region attractive for further investigation.

One candidate gene in 10q23-q25 that falls on the edge of our linkage peak (1), but beneath those reported by other groups (2,3), is the insulin-degrading enzyme (IDE) (insulysin EC 3.4.24.56). Polymorphisms in IDE have been associated with type 2 diabetes in the Goto-Kakizaki (GK) rat model (4). IDE can degrade a number of peptides, including insulin IGF-I and -II, transforming growth factor, atrial natriuretic peptide, and oxidatively damaged proteins (5).

To examine the association of IDE variants with FPG, mFPG, and $HbA_{1c,}$ we analyzed single nucleotide polymorphisms (SNPs) within and near the IDE gene in a sample of unrelated subjects and a set of families from the Framingham Heart Study (FHS) (Tables 1 and 2) (6). SNP positions and minor allele frequencies (f_a) from the November 2002 draft (University of California Santa Cruz genome browser) are given in Table 3 and shown in Fig. 1. To assess completeness of the map, linkage disequilibrium (LD) in this region was evaluated using LDMAP, a program based on the Malecot model, where one LD unit (LDU) is defined as the distance in kilobases at which the association between two markers falls by e^{-1} (Fig. 1) (7). A flat line indicates complete LD between markers and defines a haplotype block. One haplotype block is observed at the 5′ end of IDE, in agreement with a previously published report (8), and another at the 3′ end (Fig. 1). The map thus gives a reasonably complete view of the recombination history of this region in our sample.

We examined the association of SNPs with FPG (n = 1,640) and HbA_{1c} (n = 1,311) in unrelated subjects. A highly significant association was observed for rs1887922 genotypes (f_a = 0.19) for all subjects with HbA_{1c} levels ($P < 0.0001$) (Table 4). This SNP was also associated with FPG ($P = 0.03$) and mFPG ($P = 0.02$). A SNP 3′ to the IDE gene, rs2209972 (f_a = 0.37), showed a similar but less significant pattern of association with levels of FPG (P = 0.03) and HbA_{1c} ($P = 0.005$), while markers placed 5′ of the IDE gene revealed no significant association.

Stronger associations were found for rs1887922 in sex-specific analyses of unrelated men than in the pooled sample (Table 5). The results are shown in Fig. 1, where

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FHS, Framingham Heart Study; FPG, fasting plasma glucose; IDE, insulin-degrading enzyme; IPG, impaired plasma glucose; LD, linkage disequilibrium; LDU, LD unit; mFPG, mean FPG; SNP, single nucleotide polymorphism.

TABLE 1
Descriptive statistics of the 1,780 unrelated individuals by sex

Covariate	Men	Women
n	888	892
Age (years)	55.80 ± 9.64	55.35 ± 9.54
Alcohol use (oz/week)	3.59 ± 4.33	1.77 ± 2.46
Cigarettes (cigarettes/day)	3.90 ± 10.11	3.75 ± 9.29
Physical activity index	37.80 ± 8.02	36.42 ± 6.09
BMI (kg/m^2)	28.35 ± 4.17	26.79 ± 5.31
Estrogen (%)	—	19%
IPG	26%	24%
Type 2 diabetes	14.81%	11.04%

Data are means ± SD unless otherwise indicated.

the negative logarithms of *P* values [−log(p)] from Table 5 are plotted against SNP positions (Table 3). The significance of the association diminished slightly after adjustment for BMI (results not shown).

We attempted to replicate the rs1887922 results with a different sample and methodology by testing a family set using FBAT (9). Under an additive model, borderline associations were found for rs1887922 with levels of FPG ($P = 0.085$) and HbA_{1c} ($P = 0.055$). We found no significant associations with rs2209972 in the family set, as might be expected due to weaker associations observed in the unrelated sample.

To evaluate whether the particular polymorphisms in IDE accounted for the linkage previously reported (1–3), we adjusted the linkage model for the rs1887922 polymorphism. No change in either peak height or peak position was found (results not shown), suggesting that another gene present in this region accounts for the linkage signal.

We tested whether any of these common polymorphisms were associated with type 2 diabetes. When all unrelated subjects were considered, the rs1887922 SNP revealed borderline association with type 2 diabetes ($P = 0.079$) (Table 2). When sex-specific analyses of men were conducted (Table 5), a significant association with type 2 diabetes was seen ($P = 0.022$).

We also analyzed traits using haplotypes based on SNPs rs2209972 (minor allele T) and rs1887922 (minor allele C) within the sample of unrelated men. The statistical significance and directionality of association between traits and haplotypes is indicated by the Z-scores presented in Fig. 2*A* (10). The TT haplotype is associated with lower trait levels while the CC haplotype is associated with higher levels. Estimated regression coefficients for each trait

TABLE 2
Descriptive statistics of the 182 families (1,078 participants) by sex

Covariate	Men	Women
n	534	544
Age (years)	51.12 ± 10.08	52.56 ± 10.47
Alcohol use (oz/week)	3.77 ± 4.63	1.60 ± 2.56
Cigarettes (cigarettes/day)	4.19 ± 10.28	3.63 ± 8.75
Physical activity index	37.80 ± 7.77	37.11 ± 6.03
BMI (kg/m^2)	28.22 ± 4.19	27.02 ± 6.03
Estrogen (%)	—	13%
IPG	21%	18%
Type 2 diabetes	11.8%	9%

Data are means ± SD unless otherwise indicated.

TABLE 3
Marker positions on Chromosone 10 relative to IDE

					IDE (3′ to 5′) (93,435,695–93,555,489)				
Polymorphic marker	rs2901587	rs2209972	rs967878	rs884526	rs1887922	rs2275218	rs2149632	rs1999764	rs1573051
Base position	93,392,507	93,400,684	93,401,004	93,402,118	93,445,821	93,446,953	93,453,903	93,541,795	93,608,990
Distance from first marker	0	8,177	8,497	9,611	53,314	54,446	61,896	149,288	216,483
Minor allele frequency	0.26	0.37	0.5	0.34	0.19	0.05	0.35	0.1	0.35

The locations on chromosome 10 for SNPs typed in this study are given by base number using the November 2002 draft of the University of California Santa Cruz genome browser (http://genome.ucsc.edu/). The position of the IDE locus is shown. These locations were used to construct Fig. 1. The frequency of the minor allele for each SNP is given in the bottom row of the table.

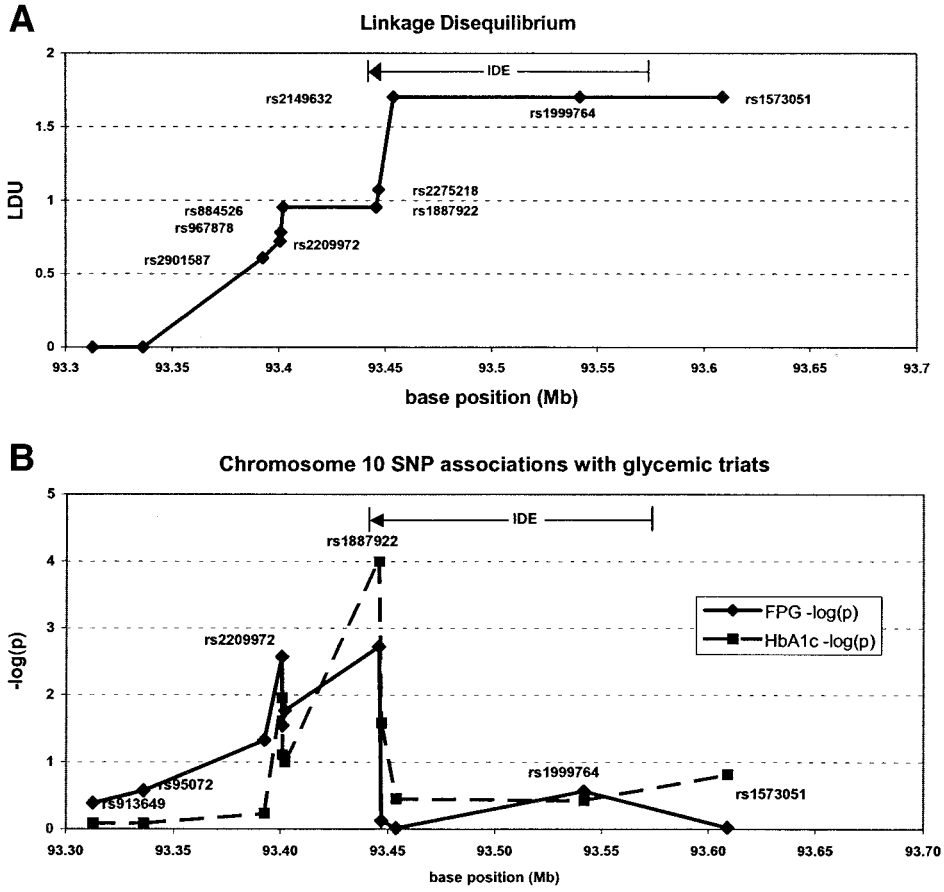

FIG. 1. Physical map of LD and SNP association for the IDE locus region. *A*: The LD estimates are made using the program LDMAP (http://cedar.genetics.soton.ac.uk/public_html/) (7). One LDU corresponds to the distance at which the association between two markers is reduced by e^{-1}. The position of IDE is shown and direction of transcription indicated by the arrow. *B*: SNP map for unrelated male subjects. Base location and SNP names are given in Table 3. These are plotted against the negative logarithm of *P* values given in Table 4. The position of IDE is shown and direction of transcription indicated by the arrow.

presented in Fig. 2*B* reveal a consistent trend with lower trait values for all traits associated with the TT haplotype and higher values with the CC haplotype.

Adding SNPs rs967878 (minor allele A) and rs884526 (minor allele T) did not improve the model, but revealed that the haplotype TATT (f~30%) was associated with lowest trait values, while the haplotype CCGC (f~16%) was associated with highest trait values (Fig. 2*C*), consistent with the two-locus analysis. The lower associations observed when SNPs rs967878 and rs884526 were analyzed individually may be explained by the presence of the major allele on haplotypes that had both high and low trait values, thus diminishing contrasts with minor allele trait values (Fig. 2*C*). Haplotype analysis was not performed in families due to the problems in obtaining estimated haplotypes in the presence of LD (11).

We also tested the association of the two-locus haplotypes with type 2 diabetes. The global model was not significant when the whole sample was tested. In contrast, global models for sex-specific analyses of men were significant and indicated that the CC haplotype was associated with increased risk of type 2 diabetes ($P = 0.001$, global-simulated P value for model = 0.014). The odds ratio for type 2 diabetes of the CC haplotype relative to the TT haplotype was 1.96 (95% CI 1.3–3.0).

To evaluate whether subjects with type 2 diabetes or prediabetes accounted for the sex-specific associations observed, men that met the criterion for impaired plasma glucose (IPG) were excluded from analysis. Significant global associations with the rs1887922 SNP and glycemic trait levels were still seen in the subsidiary male dataset for FPG ($P < 0.006$) and HbA_{1c} ($P < 0.004$) but not mFPG. Similarly, the haplotype TT was associated with low trait values for HbA_{1c} ($P = 0.009$, global P for model = 0.023) and the CC haplotype with high trait values of HbA_{1c} ($P =$

TABLE 4
Global *P* values to test the association between the three genotypes of each SNP and the traits for all unrelated subjects (men and women)

					IDE				
SNP	rs2901587	rs2209972	rs967878	rs884526	rs1887922	rs2275218	rs2149632	rs1999764	rs1573051
FPG	—	0.0362	—	—	—	—	—	—	—
mFPG	—	—	—	—	—	—	—	—	—
HbA_{1c}	—	0.0096	—	—	<0.0001	—	—	—	—
Type 2 diabetes	—	—	—	—	0.079	—	—	—	—

A general model without specific modes of transmission for genotype effects was used. Cells with — are $P > 0.05$ (FPG, n = 1,640; mFPG, n = 1,739; HbA_{1c}, n = 1,311).

TABLE 5
Global *P* values for unrelated male subjects to test the association between the genotypes of each SNP and trait values

					IDE				
SNP	rs2901587	rs2209972	rs967878	rs884526	rs1887922	rs2275218	rs2149632	rs1999764	rs1573051
FPG	0.047	0.0027	0.0286	0.0172	0.0019	—	—	—	—
mFPG	—	0.0113	0.0482	0.035	0.0006	—	—	—	—
HbA_{1c}	—	0.0111	—	—	<0.0001	0.0263	—	—	—
DM2	—	—	—	—	0.0219	—	—	—	—

Cells with a $P > 0.05$ are marked with — (FPG, $n = 822$; mFPG, $n = 876$; HbA_{1c}, $n = 626$).

0.003). These results indicate a role for the IDE locus in normal glucose homeostasis.

We thus present evidence for the association between polymorphic markers in the IDE gene on 10q23-q25 with levels of FPG, mFPG, HbA_{1c} and type 2 diabetes. The effect of rs1887922 and rs2209972 on trait levels of the individual SNPs is most pronounced in men. This may be partly due to an increased number of men with IPG in the unrelated set as compared with women. However, associations were still statistically significant following the exclusion of men with IPG from the analysis of men, suggesting that this is not the only explanation. Male-specific susceptibility has been reported in two murine models of type 2 diabetes associated with obesity (12,13). In one model, based on crosses of NON/lt and obese NZO/HI mice, a number of susceptibility genes that determine the threshold for type 2 diabetes have been mapped, none of which lie on the Y chromosome (12). Android obesity has been previously described as a major risk factor for type 2 diabetes (14), although the associations reported here persist after adjustment for BMI.

The TT haplotype, representing the minor allele of rs2209972 and the major allele of rs1887992, was associated with lowered trait values, suggesting that it encodes an IDE variant with reduced activity that increases the efficiency of insulin signaling. Less insulin would therefore be needed to reduce blood glucose levels. In contrast, the CC haplotype IDE variant may diminish insulin action, leading to higher trait values and type 2 diabetes.

The associations with HbA_{1c} and FPG map to the 3′ region of IDE and are unlikely to involve the protease domain of the enzyme, which lies at the 5′ end of the gene (5). Similarly, mutations to IDE in the GK rat model of type 2 diabetes do not affect protease activity (4). A number of alternative molecular mechanisms provide plausible explanations for delayed or diminished action of IDE. For example, a change in the 3′ untranslated region of IDE may affect the stability or the translation of the message,

A

SNP	rs2209972	T	T	C	C	Global Model
	rs1887922	T	C	T	C	
Haplotype Frequency		0.32	0.03	0.48	0.17	
FPG	Z-score	-3.111	-1.091	1.546	2.082	
	p-val	**0.001**	0.285	0.128	0.047	**0.014**
mFPG	Z-score	-2.925	-0.701	1.083	2.526	
	p-val	**0.003**	0.495	0.296	**0.015**	**0.013**
HbA_{1c}	Z-score	-2.332	-0.215	0.359	3.203	
	p-val	**0.025**	0.841	0.724	**0.002**	**0.014**
DM2	Z-score	-1.624	-0.426	0.763	3.276	
	p-val	0.094	0.678	0.460	**0.001**	**0.014**

B

SNP	rs2209972	T	T	C	C
	rs1887922	T	C	T	C
Haplotype Frequency		0.32	0.03	0.48	0.17
FPG	β –estimate	**-0.236**	-0.058	**0.160**	**0.194**
	standard error	0.084	0.209	0.060	0.075
mFPG	β –estimate	**-0.215**	0.006	0.108	**0.208**
	standard error	0.084	0.204	0.060	0.075
HbA_{1c}	β –estimate	-0.163	0.049	0.068	**0.289**
	standard error	0.092	0.214	0.067	0.085
DM2	Odds ratio	-	0.66	1.05	**1.96**
	Confidence limits	-	0.16-2.6	0.12-1.54	**1.28-3.00**

C

rs2209972	T	C	C	T	C	C
rs967878	A	A	C	A	C	A
rs884526	T	G	G	T	G	G
rs1887922	T	T	T	C	C	C
Haplotype Frequency	0.307	0.108	0.368	0.030	0.159	0.013
Haplotype Score	-2.395	-1.673	0.882	1.136	2.954	3.489
p-value	**0.016**	0.103	0.352	0.267	**0.002**	**0.000**

FIG. 2. Two and four locus haplotype analysis for the sample of unrelated men. *A*: Haplotypes were constructed using rs2207992 and rs1887922 and the association with traits (FPG, $n = 822$; mFPG, $n = 876$; HbA_{1c}, $n = 626$) determined empirically by permutation using Haplo.score (10). Global and haplotype-specific *P* values are given for each test. Values significant at $P < 0.05$ are in bold. *B*: Regression coefficient estimates (β for quantitative traits) and odds ratio estimates (for qualitative traits) measure the association of a particular haplotype with a trait (10). Values were derived using inferred haplotype probabilities for each individual as predictors. Values significant at $P < 0.05$ are in bold. *C*: Haplotypes based on a four-locus model for HbA_{1c} values (global $P = 0.002$). The output score from Haplo.score (10) is shown. The lower associations, observed when SNPs rs967878 and rs884526 were analyzed individually, can be explained by the presence of the major allele on haplotypes with both high and low trait values, thus diminishing the contrast with minor allele trait values.

altering levels of IDE protein produced. Alternatively, coding polymorphisms in the carboxyl terminal region of the protein may modify cellular localization, homodimerization (15), or interaction with protein partners.

Other genes on 10q23-q25 appear to contribute to linkage of this region reported previously by others and us with glycemic traits (1–3), as the signal for FPG was not diminished by adjustment for the rs1887922 SNP. Association studies may be more efficient than linkage studies in identifying genetic determinants of complex traits when allele frequency is relatively high and the effect small (16). This finding is consistent with the associations we see here with the IDE CC haplotype (f~16%), which is about twice as likely to be associated with type 2 diabetes as the TT haplotype. Typing of additional SNPs will be necessary to identify other gene variants on 10q23–25 that affect glucose homeostasis.

It has been proposed that typing one SNP per haplotype block will be sufficient for genome-wide association studies (17,18). Here, we show that two SNPs rs1887922 and rs884526 on the same haplotype block vary in association with trait values due to the different haplotypes on which their minor allele is found. Typing only one SNP per haplotype block may not be sufficient in all cases to reliably detect or exclude associations of candidate loci with complex diseases.

RESEARCH DESIGN AND METHODS

Subjects. We used subjects from two samples: *1*) a group of unrelated subjects from the Framingham Offspring Cohort recruited in the early 1970s, and *2*) a subgroup of 182 families coming from the largest 330 pedigrees in the FHS. The latter group contains the majority of those included in the genome scan results reported in Meigs et al. (1). Descriptive statistics are presented in Tables 1 and 2. This study was approved by the Boston University IRB, and written informed consent was obtained from each subject.

Traits. Trait measurements have been previously described (1). For the sample of unrelated subjects, FPG, measured at exam cycle 5, was available for 1,640 Offspring participants. The mFPG was the average FPG value for the 1,739 offspring who had three or more measures of FPG in the five exams that spanned 20 years. HbA_{1c} was determined for 1,311 individuals at exam 5. In the family set, FPG was available for 837 individuals, mFPG for 951 individuals, and HbA_{1c} for 528 individuals. Diabetes status (type 2 diabetes) was defined as either on hypoglycemic medication or two FPG levels ≥7 mmol/l (>125 mg/dl) at any time during exams 1–6. Using this criterion, 14.8% of men in the unrelated sample and 11% of women were classified as diabetic subjects (Tables 1 and 2). A less stringent criterion for IPG was defined as FPG ≥6.1 mmol/l (>110 mg/dl) or 2-h postchallenge plasma glucose ≥7.8 mmol/l (>140 mg/dl) or anyone being treated for type 2 diabetes. A questionnaire was used to collect information on alcohol and cigarette consumption, hormone therapy, and physical activity index, which is calculated as a weighted average of hours spent at various activity levels (19).

Genotyping. SNPs were scored with a Sequenom MALDI-TOF mass spectrometer (20).

Statistical analysis. Standardized residuals were calculated using linear regression models that incorporated age, age-squared, age-cubed, sex, smoking, physical activity, BMI, and alcohol consumption as covariates. Separate regression models were used for men and women. The residuals from the models were then ranked and normalized (1). This transformation diminished slightly the significance of the results reported. For the sample of unrelated subjects, associations between traits and genotypes for each SNP were evaluated using regression models. Additionally, association with haplotypes was tested using Haplo.score (10) and haplotype effects were estimated using the REGRESSION and LOGISTIC procedures in SAS. Haplotype effects were estimated in a two-stage procedure. First, individual haplotypes were inferred by the EM algorithm using *snphap* (D. Clayton, http://www-gene.cimr.cam.ac.uk/clayton/software/); then, the inferred haplotype probabilities were used as predictors in regression models. Effect measures from these analyses compare each haplotype against all other haplotypes. The global significance of the haplotype-phenotype associations were determined using empirical *P* values (1,000 permutations) obtained from Haplo.score (10). In related individuals, a family based (empirical) test of association implemented in the program FBAT was used with an additive model (9).

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