

Quantitative Linkage Analysis for Pancreatic B-cell Function and Insulin Resistance in a Large Twin Cohort

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OBJECTIVE—Insulin resistance and disturbed glucose homeostasis are key characteristics of metabolic syndrome, diabetes, and cardiovascular disease. The recent nonlinear computer version of homeostasis model assessment (HOMA)2 provides an appropriate and convenient assessment of glucose metabolism, enabling gene-mapping studies in large population samples.

RESEARCH DESIGN AND METHODS—Fasting insulin and glucose concentration were measured in 758 dizygous and 305 monozygous nondiabetic female pairs from the St. Thomas' U.K. adult twin registry (TwinsUK). Insulin resistance (IR) and pancreatic β -cell function (BCF) were estimated from this data using the HOMA2 model.

RESULTS—Genome-wide variance component linkage analysis using 2,231 genetic markers identified a highly significant quantitative trait locus for BCF on chromosome 10p15 (logarithm of odds [LOD] 6.2, $P = 0.0001$), a region recently shown to contain a functional variant for type 1 diabetes. Both BCF and IR suggested a pleiotropic effect on 17q25 (univariate LOD 3.2, $P = 0.0012$, and 2.38, $P = 0.0087$; bivariate LOD 2.66), and one additional region showed linkage for IR on chromosome 22q11 (LOD 3.2, $P = 0.0016$), providing replication and refining previous findings for diabetes and associated traits.

CONCLUSIONS—To our best knowledge, this is the first genome-wide linkage screen for HOMA2 indexes in a large, healthy female sample. These results suggest that loci involved in control of normal glucose homeostasis among nondiabetic individuals might overlap with those involved in the development of diabetes. Linkage replications in independent studies and across populations provide information on important regions of common but potentially heterogeneous variability that can now be used for targeted positional candidate studies. *Diabetes* 57: 1120–1124, 2008

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BCF, β -cell function; HOMA, homeostasis model assessment; IR, insulin resistance; QTL, quantitative trait loci.

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Type 2 diabetes in both obese and nonobese individuals is characterized by insulin resistance (IR) and defective pancreatic β -cell function (BCF) (1,2). The metabolic syndrome and cardiovascular disease in some individuals share similar aspects of defective glucose metabolism (3). Disturbance of glucose homeostasis is also a risk factor for the progression and susceptibility to type 1 diabetes (4,5).

Historically, the stimulated euglycemic clamp has been regarded as the optimum method for quantitative estimate of dynamic insulin sensitivity and determination of pancreatic BCF, but the technique is impractical to apply in large population studies and provides information different from that of steady-state techniques. Methods such as the homeostasis model assessment (HOMA) (6) estimate BCF and IR under steady-state conditions and have proven to be robust and easy to apply for large cohort studies and highly correlated with other methods such as stimulated euglycemic clamp (7). The HOMA model was later improved using a more advanced nonlinear computer model (HOMA2) (8) that provides a more accurate representation of physiology. In this study, we performed a genome-wide screen for HOMA2 indexes on a large cohort of unselected female twins to identify regions of the genome that contain quantitative trait loci (QTL) that regulate normal glucose homeostasis.

RESEARCH DESIGN AND METHODS

Female twins identified from the St. Thomas' U.K. adult twin registry (TwinsUK) were invited to participate in a study of common diseases and traits (9). General medical and lifestyle data were obtained by questionnaire. Plasma glucose and insulin were measured for 2,126 individuals (758 dizygous and 305 monozygous pairs) after an overnight fast of 10 h. Insulin was measured by immunoassay (Abbott Laboratories, Maidenhead, U.K.) and glucose by an Ektachem 700 multichannel analyzer using an enzymatic colorimetric slide assay (Johnson and Johnson Clinical Diagnostic Systems, Amersham, U.K.), as described previously (10).

HOMA2 software (<http://www.dtu.ox.ac.uk/homa>) was used for the calculation of IR (HOMA2-IR) and BCF (HOMA2-%B). Individuals who were hypoglycemic (glucose <2.5 mmol/l), those on lipid- or blood glucose-lowering medications, and pairs in whom at least one sib was diagnosed with type 2 diabetes were excluded from the study.

Approval for the research protocols was obtained from the St. Thomas' Hospital Institutional Ethics Committee.

Genotyping. Genome scans were performed using DNA from leukocytes. Scans involved analysis of 737 microsatellite markers from the ABI Prism set (Applied Biosystems, Foster City, CA), as described previously (11), and 1,494 single nucleotide polymorphism markers from the HuSNP GeneChip linkage mapping set (Affymetrix, Santa Clara, CA), providing approximate inter-marker spacing of <10 cM. Twin zygosity and family relationships were rigorously investigated and discrepant pairs discarded from further analyses. The estimated genotyping error rate was <1%.

Allele frequencies were estimated from the whole sample of genotyped subjects. The map positions were taken from the Rutgers Combined Linkage-

TABLE 1

Median (25–75% quartiles) of BMI, glucose, insulin, β -cell function, and insulin sensitivity (inverse of IR, for clarity) for all 2,126 twins of the TwinsUK registry used in the analysis and for the two subgroups above and below the median age

Age class	<i>n</i>	BMI (kg/m ²)	Glucose (mmol/l)	Insulin (pmol/l)	HOMA2-%B	HOMA2-IS
<50	1,074	23.9 (21.5–26.9)	4.3 (4.1–4.6)	43.8 (32.6–59.7)	110.9 (90.5–137.5)	126.8 (92.8–169.7)
>50	1,052	25.6 (23.1–28.7)	4.6 (4.3–5.0)	46.5 (33.3–66.7)	103.2 (81.0–130.3)	117.9 (81.3–165.1)
All	2,126	24.8 (22.3–28.0)	4.5 (4.2–4.8)	45.1 (33.3–63.2)	107.3 (85.8–134.5)	121.3 (87.4–168.0)

Data are median (25–75% quartile range).

Physical Map of The Human Genome (12,13) or interpolated from their physical position.

Statistical analysis. Clinical data were analyzed using R (R Foundation for Statistical Computing, Vienna, Austria) (14). To maximize the closeness of the data to normality, we applied a Box-Cox transformation using the `box.cox.power` function in the `car` package (15,16), which reduces type I errors while preserving power for the linkage analysis of skewed distributed phenotypes (17). The Box and Cox transformation is defined as:

$$\tau(X; \lambda) = \begin{cases} (X^\lambda - 1)/\lambda & \text{if } X \neq 0 \\ \log(X) & \text{if } X = 0 \end{cases}$$

and includes, as special cases, all of the transformations in common use, including reciprocals, logarithms, and square roots. The purpose of transformation is to raise the data to power λ , whose value is evaluated through maximum likelihood. Phenotypic values falling outside the mean ± 3 SDs were excluded from the analysis.

Univariate multipoint variance component linkage analyses were performed using Merlin (18). A linear mixed model was fitted to the data so that the phenotypic variance of the trait mean was partitioned into a monogenic component representing the contribution of a QTL, a polygenic component attributable to residual additive genetic variance, and a residual component attributable to environmental effects. The phenotypic variance/covariance in sibs was modeled using the expected proportion of alleles shared identical by descent (IBD) over the genome to estimate the polygenic component as well as the proportion of alleles shared IBD estimated from the genotypic data at a point in the genome to estimate the QTL effect. Parameters were estimated by maximum likelihood under the assumption of multivariate normality (19). Approximate support intervals were generated using a -1 logarithm of odds (LOD) approach.

Empirical *P* values were derived with Merlin by gene dropping, simulating 10,000 datasets for every significantly linked region (which included all the markers within the interval showing LOD >0) while retaining the phenotypic information and observing the number of times the observed LOD was exceeded by chance within the whole regions. Where correlated traits mapped to the same chromosomal location, we evaluated bivariate linkage analysis as implemented in SOLAR (20,21). In the bivariate linkage model, the phenotypic covariance is further decomposed to include the correlation between traits caused by the QTL, residual additive genetic effects, and random environmental effects. To test pleiotropy and coincident linkage, we used the method proposed by Almasy et al. (21).

RESULTS

The phenotypic characteristics of the 2,126 female subjects used in the analysis are summarized in Table 1. Pairwise correlations among the variables used in this analysis are shown in Table 2. The median (25–75% quartiles) age of twins was 50 years (range 38–59). Individuals after the age of 50 years had increased mean insulin, glucose, and HOMA2-IR and decreased HOMA2-%B (Mann-Whitney using random singletons, $P < 0.001$). The median

TABLE 2

Correlation coefficients among the traits used in this study

	HOMA2-IR	Insulin	Glucose	BMI
HOMA2-%B	0.68	0.72	–0.3	0.2
HOMA2-IR		0.99	0.4	0.24
Fasting insulin			0.35	0.24
Fasting glucose				0.17

(25–75% quartiles) BMI was 25 kg/m² (range 22–28). Higher BMI values were associated with increased insulin, glucose, HOMA2-IR, and HOMA2-%B (Mann-Whitney using random singletons, $P < 0.001$).

HOMA2-%B and HOMA2-IR distributions were positively skewed. The maximum likelihood estimates of the λ parameter for the Box-Cox power transformation was -0.116 for HOMA2-%B and -0.575 for HOMA2-IR.

The dizygous correlations for HOMA2-%B and HOMA2-IR (0.51 and 0.56, respectively) were lower than the monozygous correlations (0.68 and 0.88), highlighting that genetic factors are operating on both traits. Heritability estimates were 0.63 for HOMA2-%B and 0.58 for HOMA2-IR, using age and BMI as covariates.

With a sample of 758 sibpairs, we have $\sim 80\%$ power to detect a QTL (at $\alpha = 0.0001$) that explains 30% of these trait variations, assuming a recombination fraction of 0.0 (22). Genome-wide variance component analysis (see online appendix [available at <http://dx.doi.org/10.2337/db07-0708>] incorporating correction for age and BMI identified evidence of linkage for HOMA2-IR on chromosome 22q11.22-q12.2 with peak LOD 3.2 (Fig. 1C) at marker D22S1167. HOMA2-%B showed evidence of linkage to two chromosomal regions, 10p15.1-2 with peak LOD 6.2 (Fig. 1A) at marker D10S1713 and 17q25.1-3 with peak LOD 3.2 (Fig. 1B) at marker D17S785. This latter region also showed a linkage signal for HOMA2-IR with peak LOD of 2.38 at the same D17S785 marker. Suggestive linkage (23) was observed for HOMA2-%B on chromosome 18p11.1-21 with peak LOD 2.1 at marker D18S464. Table 3 reports the LOD findings, support intervals, and empirical *P* values for both phenotypes. The empirical *P* values, which are region wise, since they have been evaluated considering all the markers within each linkage peak, suggest that the observed linkage signals may be slightly inflated. Similar results were obtained when the analysis was performed without adjustment for BMI, apart from the linkage peak on chromosome 17, which decreased to 1.73 for HOMA2-%B and to 1.1 for HOMA2-IR.

Bivariate analysis indicated significant genetic (0.7 ; $P < 10^{-6}$) and environmental (0.7 ; $P < 10^{-6}$) correlations between the two phenotypes. Bivariate linkage analysis incorporating correction for age and BMI was evaluated on 17q25.1-3, where linkage was observed for both traits. The maximum LOD from bivariate analysis was 2.66 (QTL-specific correlation 0.88; residual polygenic correlation 0.23). We rejected the hypothesis of coincident linkage ($P = 0.0002$) but failed to reject the hypothesis of pleiotropy ($P = 0.53$).

DISCUSSION

Shifts in insulin sensitivity are commonly accompanied by compensatory alterations in β -cell sensitivity to glucose and vice versa. Environmental factors and genetic predisposition might alter the maintenance of

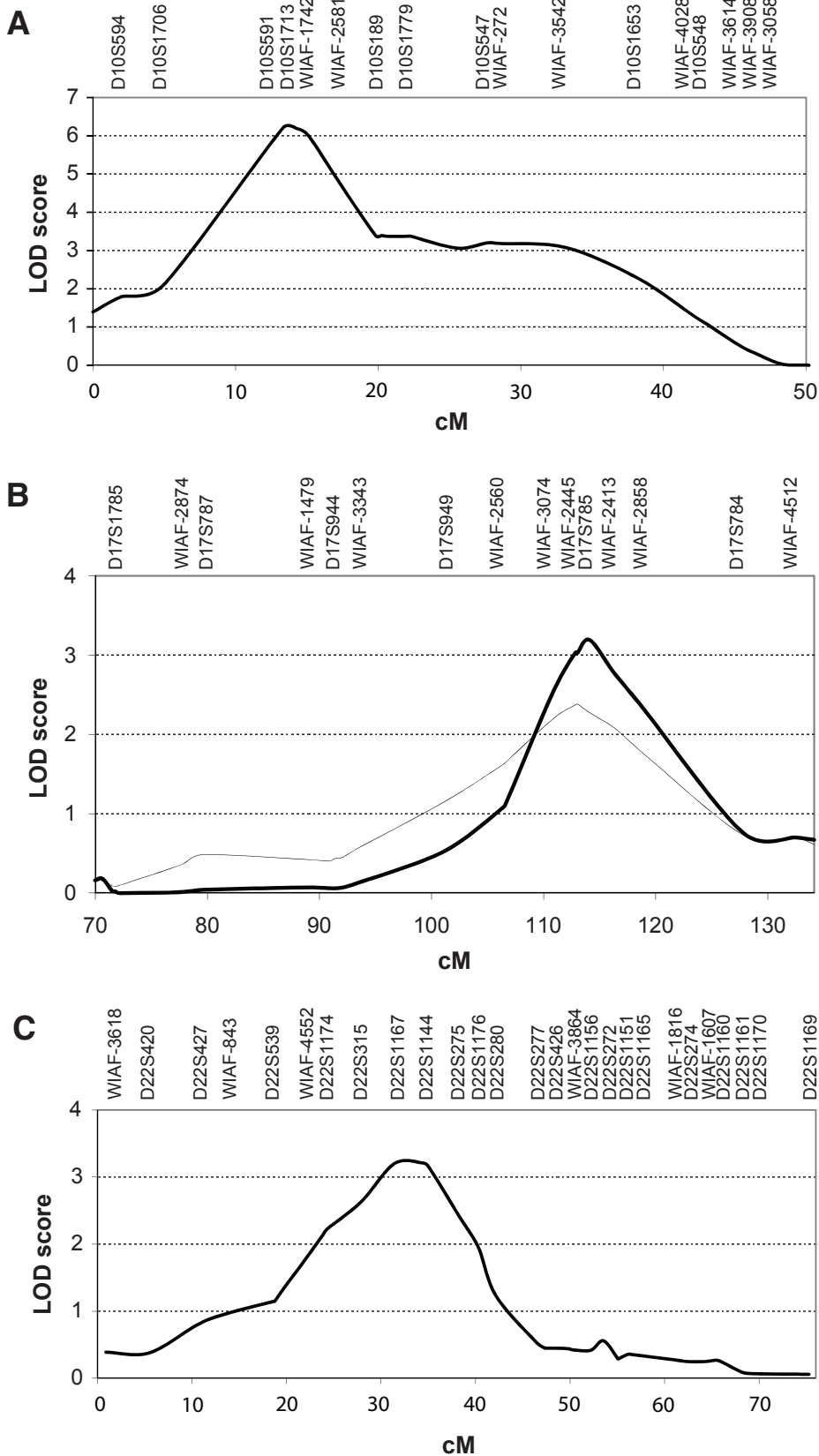


FIG. 1. Major linkage peaks for HOMA2-%B on chromosome 10 (A) and 17 (B, thick line) and HOMA2-IR on chromosomes 17 (B) and 22 (C) (thin lines) obtained by genome-wide variance component linkage analysis, incorporating age and BMI as covariates.

glucose homeostasis, causing abnormalities in the equilibrium between insulin secretion and action, which are risk factors for metabolic syndrome, diabetes, and cardiovascular disease. An efficient way to identify genes responsible for these heterogeneous disorders is to focus on the

genetic causes of IR and the failure of adequate β -cell compensation.

We estimated BCF and IR by the application of the newer nonlinear HOMA2 model to investigate the genetic components responsible for physiological varia-

TABLE 3
Multipoint linkage results for HOMA2-IR and HOMA2-%B

Chromosome	Trait	Peak marker	Support interval (cM)	LOD	Empirical <i>P</i>
17q25.1-3	HOMA2-IR	D17S785	110.67–131.72	2.38	0.0087
22q11.22-q12.2	HOMA2-IR	D22S1167	22.13–38.49	3.2	0.0016
10p15.1-2	HOMA2-%B	D10S1713	6.28–17.84	6.2	0.0001
17q25.1-3	HOMA2-%B	D17S785	110.67–131.72	3.2	0.0012
18p11.1-21	HOMA2-%B	D18S464	19.18–37.08	2.1	0.0070

tions in our sample. Median insulin and glucose data were significantly higher in older subjects, even when within acceptable clinical ranges. This translates to a decreased HOMA2-%B and increased HOMA2-IR with increasing age, creating a potentially pathological combination.

Significant positive correlation was also observed between BMI and both median insulin and glucose levels. Thus, increments in BMI are associated with increased HOMA2-%B and HOMA2-IR in our data. The observation of an apparently matched increase in β -cell activity in the presence of increasing insulin resistance may help to explain why a proportion of individuals with BMI > 30 kg/m² in the community, who are not morbidly obese, lack most of the metabolic abnormalities typical of the metabolic syndrome and type 2 diabetes.

Quantitative genetic analysis showed that additive genetic effects accounted for 63 and 58% of total HOMA2-%B and HOMA2-IR variability. The genome-wide search replicated linkage to chromosomal regions already highlighted by other studies for disturbed glucose homeostasis and related diseases. For HOMA2-%B we identified a highly significant QTL on chromosome 10p15.1-2 and observed two further linkage signals on 17q25.1-3 and 18p11.1-21.

The 10p15 region has been recently studied by Vella et al. (24) in a type 1 diabetes case-control association study with the candidate gene *IL2RA*. The authors replicated the association in a family sample and suggested that a functional variant is likely to be present in the *IL2RA* gene or in a flanking gene in linkage disequilibrium with *IL2RA*. Precisely the same region has also been linked to BMI and fat mass in the HERITAGE study (25) and BMI in a study of Pima Indians (26), with the gene *AKR1C2* identified as a possible candidate for these latter phenotypes. Our data localizing this strong QTL, which appears to determine BCF variability in our sample, should help in pinpointing the functional variant(s) and in determining which specific phenotypes are regulated.

Chromosome 17q25 showed linkage also with HOMA2-IR. Promising linkage in the same region has been recently observed for HOMA2-IR in a Caucasian ascertained sample of hypertensive subjects (27) and in sibpairs affected with type 2 diabetes (28). This region contains the *GCCR* gene already associated with type 2 diabetes in a pooled French and Sardinian sample (29). Given the significant genetic correlation (0.7; $P < 10^{-6}$) between the two traits, the chromosome 17q25 region was analyzed in a bivariate framework obtaining LOD of 2.66, slightly less significant than the maximum univariate LOD at the same location. To gain from the increase in power of bivariate linkage analysis, the genetic correlation at the QTL and the residual correlation must be of different signs (30), which was not the case here. Nevertheless, while the hypothesis of coincident linkage was rejected, we failed to reject the hypothesis of pleiotropy, suggesting that a QTL located in this region may exert an effect on both phenotypes.

The linkage signal on 18p11 for HOMA2-%B has been replicated in previous linkage studies of type 2 diabetes in the Dutch and Finnish/Swedish populations (28,31,32) and for metabolic syndrome (33). A recent functional mapping across genes in 18p11 suggested the *LAMA1* gene to have a role in pancreatic BCF (34).

The highest evidence of linkage for HOMA2-IR was observed on 22q11.22-q12.2; this region has been repeatedly linked to type 2 diabetes (35,36). The support interval contains many genes, among these *XBPI*, with a potential role in regulation of glucose homeostasis from studies of mice (37).

The HOMA2 indexes have proven to be clinical and epidemiological robust and compare favorably with other well-validated methods for the measure of IR and BCF, where steady-state conditions are maintained (i.e., nondiabetic individuals, as in this study) (7). The concordance of our linkage data with relevant phenotypes from other studies lends support to the use of HOMA2 for the characterization of IR and BCF in large cohort studies.

These twins have been shown to be comparable with age-matched singleton populations in terms of disease-related and lifestyle characteristics (38), though we cannot extrapolate these data to male subjects. Genotyping is planned to further dissect the allelic architecture of these loci.

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