A genome scan for loci linked to quantitative insulin traits in persons without diabetes: the Framingham Offspring Study

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Abstract

Aims/hypothesis. Hyperinsulinaemia and insulin resistance usually precede clinical hyperglycaemia and Type 2 diabetes. Thus, plasma insulin concentrations and insulin resistance are important quantitative traits associated with risk of Type 2 diabetes, and represent key measures for genetic analysis of the syndrome.

Methods. We carried out a genome-wide search for loci related to plasma insulin concentrations and insulin resistance in 330 extended, community-based pedigrees from the Framingham Heart Study. Normalized deviates of the standardized residuals of plasma insulin concentrations in the fasting state, 2 h after oral glucose challenge and as a measure of insulin resistance were used in linkage analysis with the variance components model implemented in the computer program SOLAR.

Results. The results suggest susceptibility loci influencing plasma concentrations of fasting insulin and

insulin resistance on chromosomes 11 (LOD 2.43 at 85 cM close to D11S2002) and 17 (LOD 1.8 at 60 cM, close to D17S784); and susceptibility loci influencing 2-h plasma insulin concentrations on chromosomes 9 (LOD 2.8 at 80 cM, close to D9S922) and 19 (LOD 1.8 at 66 cM, close to D19S245). The results of the analysis of 1000 simulations of the trait and an unlinked marker suggest that in a genome scan of 401 markers fewer than one LOD score over 1 would be due to Type 1 error, and be a false positive.

Conclusion/interpretation. We conclude that these suggestive regions for quantitative pre-diabetic insulin traits could contain major loci in the pathogenesis of Type 2 diabetes. [Diabetologia (2003) 46:579–587]

Keywords Insulin, insulin resistance, diabetes mellitus, non-insulin-dependent, chromosome mapping, chromosomes, human, pair 9, chromosomes, human, pair 11, chromosomes, human, pair 17.

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Abbreviations: DM, Diabetes mellitus; IR, Insulin resistance; HOMA-IR, homeostasis model-assessed insulin resistance (fasting insulin (uU/ml)×fasting glucose (mmol/l))/22.5; LOD, logarithm of the odds ratio of linkage between a chromosomal locus and a trait; NPL, non-parametric LOD score; QTL, quantitative trait locus.

Type 2 diabetes is a common metabolic disorder affecting about 6 to 7% of the US population and is increasingly common worldwide. It is defined by high plasma concentrations of plasma glucose in either the fasting state or after an oral glucose challenge [1]. Development of hyperglycaemia and Type 2 diabetes is characterized by tissue resistance to insulin action and, despite compensatory hyperinsulinaemia, failure of pancreatic beta cells to produce plasma concentrations of insulin adequate to overcome peripheral insulin resistance (IR) [2]. It is widely accepted that IR is an early finding, evident before the onset of hyperglycaemia and predictive of the development of Type 2 diabetes [3]. As such, plasma concentrations of insulin and of IR are important quantitative traits associated with risk for developing Type 2 diabetes, and re-

present powerful measures in the genetic analysis of the syndrome.

Several genome-wide scans have been carried out on prediabetic insulin traits. In most studies however, the families and participants were collected through an index patient with Type 2 diabetes, and the study cohorts were known to have a higher risk for developing Type 2 diabetes, such as Pima Indians, Mexican Americans and Australian Aborigines. In unaffected individuals three genome scans carried out from such higher risk populations resulted in several candidate regions on chromosome 3 [4, 5], chromosome 4 [4], chromosome 9 [4], chromosome 10 [6], chromosome 13 [6], chromosome 17 [6] and chromosome 22 [4]. However, probably due to the complex nature of the disease, failure of replication of postulated genes and false-positive discovery of new loci remains a major concern.

We provide results of a genome-wide search for loci associated with plasma concentrations of insulin and IR (expressed as homeostasis model-assessed IR, or HOMA-IR) from an unselected, low diabetes-risk cohort including 330 extended, community-based pedigrees from the Framingham Heart Study [7].

Subjects and Methods

Subjects. Participants of the Framingham Offspring Study are the natural children of the original Framingham Heart Study families. At the first Offspring Study examination from 1971 to 1975 participant ages ranged from 12 to 58 years. In 1995, at the 5th examination, 2607 offspring in 1643 pedigrees completed 20 years of quadrennial follow-up, with the exception of 8 years between the first and second exams. These subjects underwent a clinical and fasting laboratory examination, with written informed consent obtained prior to examination. The Boston University Institutional Review Board approved the study for Human Subjects Research.

In this analysis we included members of 330 extended pedigrees, consisting of 1444 nuclear families, (original Cohort parents with offspring), in whom 1815 individuals were genotyped by the Marshfield Mammalian Genotyping Service. Of these, 113 individuals with familial or genotyping errors were excluded, leaving 1702 individuals genotyped, of whom 394 were original cohort members and 1308 were offspring study subjects.

Among offspring subjects with genotype data, 1214 subjects (600 men and 614 women) attended exam 5 and provided phenotype data. Due to missing values in the independent parameters used in adjustment of insulin traits, the final analysis included 1158, 1134, and 1157 offspring subjects contributing data on plasma concentrations of fasting insulin, insulin at 2 h after an oral glucose challenge (2-h insulin), and HOMA-IR respectively. Although phenotypic trait data were not available from original cohort subjects, 394 of them contributed genotypic information to the IBD sharing probabilities in this analysis.

Subjects previously diagnosed and treated for diabetes were excluded from the analysis, since diabetes treatment directly affects the insulin measures and could cause subjects who are carriers of one or more diabetes susceptibility loci to have insulin plasma concentrations within the normal range, thereby

masking evidence for linkage. Diabetes was defined by self-reported hypoglycaemic drug therapy or by two or more fasting plasma glucose concentrations greater than or equal to 7.0 mmol/l at any exam (1–5) [8].

Covariates. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Smoking was assessed as the number of cigarettes smoked on average per day during the year before the examination (cigs/day). Physical activity was assessed as a weighted sum of the number of hours of a typical day spent sleeping and doing sedentary, slight, moderate, or heavy physical activities [9]. Alcohol use was categorized as usual consumption in ounces of alcohol per week (oz/wk). Estrogen use (either birth control or estrogen replacement therapy) was defined as present or absent.

Metabolic testing. Fasting insulin was measured in EDTA plasma as total immunoreactive insulin (Coat-A-Count Insulin, Diagnostic Products, Los Angeles, Calif., USA). Cross-reactivity of this assay with pro-insulin at mid-curve is approximately 40%, the intra-assay and inter-assay CV ranged from 5.0% to 10.0% for concentrations reported here, and the lower limit of sensitivity was 1.1 $\mu\text{U/ml}$ (8 pmol/l). Plasma glucose was measured with a hexokinase reagent kit (A-gent glucose test, Abbott, South Pasadena, Calif., USA). Glucose assays were run in duplicate and the intra-assay coefficient of variation ranged from 2% to 3%, depending on the assayed glucose concentration.

HOMA-IR was calculated as $[\text{fasting plasma insulin (uU/ml)} \times \text{fasting plasma glucose (mmol/l)}] / 22.5$ [10].

Concentrations of fasting insulin and HOMA-IR both provide reliable estimates of IR across the range of glucose tolerance test with minimal model analysis or the glucose clamp technique as the gold-standard measure of insulin sensitivity [11, 12]. Fasting hyperinsulinaemia and increased HOMA-IR are powerful predictors of increased risk for glucose intolerance [13, 14, 15]. Hyperinsulinaemia 2 h after an oral glucose challenge is a characteristic of impaired glucose tolerance and also confers increased risk for Type 2 diabetes [13]. As such, the insulin measures considered here represent valid quantitative traits for genetic analysis of Type 2 diabetes.

Statistical methods. Descriptive statistics were computed using means and standard deviations for continuous measurements. A p value less than 0.05 was considered statistically significant. Linear regression models were used prior to linkage analyses to adjust the insulin traits for potentially confounding covariates, including age, smoking, alcohol use, physical activity and estrogen usage in women. Since there is a known difference in these confounders between genders separate regression models were used for men and women. The residuals from these models thus reflect deviations from the mean value for subjects in the cohort with similar covariates. These residuals were used for the linkage analyses with men and women combined. Separate models were constructed with or without a term for BMI.

Linkage analyses were carried out on the normalized deviates derived from the ranked standardized residuals for concentrations of fasting insulin, 2-h insulin and HOMA-IR to avoid the potential for false positives induced by analysis of the skewed data distributions. We constructed models with and without covariate adjustment and with and without adjustment for BMI in order to show the effect of phenotype manipulation on residual polygenic variance. Models not including BMI were run based on the hypothesis that obesity might be in the causal pathway leading to Type 2 diabetes. In that situation adjusting for values of BMI would obscure evidence for linkage to a specific locus.

Table 1. Characteristics of all offspring subjects included in the analysis

	Women		Men		<i>p</i> -value
	<i>n</i> *	Mean (SD)	<i>n</i> *	Mean (SD)	
Age (in years)	614	51.9 (10.1)	600	51.2 (10.0)	0.22
BMI (in kg/m ²)	614	26.9 (5.9)	600	28.1 (4.0)	<0.001
Fasting plasma insulin (in µU/ml)	584	27.6 (9.7)	574	31.1 (12.9)	<0.001
2-h insulin (in µU/ml)	575	92.5 (59.5)	559	92.3 (70.2)	0.09
HOMA-IR	584	6.6 (3.6)	573	7.8 (4.5)	<0.001
Smoking** (in cigs/day)	120	20.0 (3.4)	121	22.6 (5.6)	0.09
Alcohol** (in oz/wk)	396	2.6 (2.8)	461	4.6 (4.9)	<0.001

* *n*: number of individuals, SD: standard deviation

** Self-reported; only subjects responding affirmative were listed

Genotyping. Genomic DNA was extracted from peripheral lymphocytes using a Qiagen Blood and Cell Culture DNA Maxi Kit (Qiagen, Valencia, Calif., USA). The Marshfield Mammalian Genotyping Service carried out a genome-wide scan. The set of 401 microsatellite markers [16] covers the genome at an average density of one marker every 10 cM and has an average heterozygosity of 0.77 (Screening Set v. 8) [17]. The screening set and genotyping protocols are available at the website of the Center for Medical Genetics, Marshfield Medical Research Foundation (<http://research.marshfieldclinic.org/genetics/>). Map distances were assumed according to the Marshfield map.

Genetic analysis. Familial relationships were checked using ASPEX, and random genotyping errors were checked using Gentest, a precursor to the INFER procedure, in PEDSYS. We carried out linkage analysis using the variance component model implemented in SOLAR (Sequential Oligogenic Linkage Analysis Routines) for quantitative traits. As the insulin quantitative trait distributions were skewed and the variance component method is sensitive to deviations from the normal distribution, we obtained normalized deviates from the standardized residuals from multivariate linear regression models, thereby forming perfectly normally distributed measures. The variance component model in SOLAR makes no assumptions about the mode of transmission and uses all of the data from the pedigree without breaking it up into smaller subsets [18]. The results are expressed in LOD scores, the logarithm of the odds ratio of linkage between a chromosomal locus and a trait. A LOD score of at least 1.9 marks a region with suggestive linkage while a LOD score over 3.3 is considered evidence of genome-wide significance [19].

We further evaluated the potential for obtaining false-positive evidence for linkage by deriving the distribution of nominal LOD scores under the null hypothesis of no linkage by simulation using the SIMQTL script implemented in SOLAR. We simulated an unlinked marker using our pedigree structure and assigned trait values for each derived trait to those subjects in our pedigrees that had the observed traits. Since the pedigree structure for the traits fasting plasma insulin and HOMA-IR are almost identical the simulations would be identical too. For this reason only simulations for fasting plasma insulin and for 2-h insulin concentrations were carried out. These newly-created unlinked data sets were analyzed with a two-point linkage analysis to generate an expected LOD score distribution under the null hypothesis. We repeated this procedure 1000 times for both simulated traits and estimated the proportion of samples that had LOD scores greater than one, two or three. This strategy can be used to evaluate the potential proportion of false positives given the observed pedigree structure.

Table 2. Heritability estimates

Trait	Heritability	Std Error	<i>n</i>
Fasting Insulin			
– Crude	0.51	0.073	1158
– Age, sex adjusted	0.49	0.073	1158
– Age, sex and BMI adjusted	0.48	0.073	1158
– Fully adjusted w/o BMI*	0.43	0.071	1158
– Fully adjusted*	0.46	0.072	1158
2-h Insulin			
– Crude	0.54	0.074	1134
– Age, sex adjusted	0.47	0.073	1134
– Age, sex and BMI adjusted	0.47	0.072	1134
– Fully adjusted w/o BMI*	0.47	0.072	1134
– Fully adjusted*	0.47	0.072	1134
HOMA-IR			
– Crude	0.52	0.074	1157
– Age, sex adjusted	0.49	0.072	1157
– Age, sex and BMI adjusted	0.45	0.073	1157
– Fully adjusted w/o BMI*	0.42	0.071	1157
– Fully adjusted*	0.44	0.073	1157

* Fully adjusted; insulin traits were adjusted for potentially confounding covariates, including age (with squared and cubic terms to allow for non-linearity), sex, smoking, alcohol use, physical activity, estrogen usage in women. Separate models were constructed with or without a term for BMI

Results

A total of 330 pedigrees consisting of 1444 nuclear families with 4692 subjects were analyzed (Table 1). Of all subjects fitting the inclusion criteria, 1158 individuals had measures of fasting plasma insulin concentrations, 1134 had 2-h plasma insulin concentrations and from 1157 subjects a HOMA-IR value could be calculated. Phenotype manipulation had a minimal effect on the estimated heritability for all three traits. The heritability was at the most 10% higher in the ranked crude values as compared to the adjusted traits. For fasting insulin, 2-h insulin and HOMA-IR heritability was 0.4 or greater, suggesting that these traits have a substantial genetic basis (Table 2).

Table 3. Maximum multipoint LOD scores for normalized deviates of fasting insulin, 2-h insulin and HOMA-IR (not adjusted for BMI) listed by chromosome and position in cM. The LOD scores over 1.9 reflecting suggestive linkage are highlighted

Chr	Fasting Insulin			2-h Insulin			HOMA-IR		
	LOD	cM	Close to Marker(s)	LOD	cM	Close to Marker(s)	LOD	cM	Close to Marker(s)
1	1.0285	207	D1S518–D1S1660	0.653	75	D1S2134	1.2085	224	D1S1663
2	0.8594	1	D2S2976	1.0416	0	D2S2976	0.6673	0	D2S2976
3	1.1923	212	D3S2398–D3S2418	0.313	185	D3S3053–D3S2427	1.3019	211	D3S2398
4	0.8231	139	D4S1644	0.5663	195	D4S408	1.0857	136	D4S2394–D4S1644
5	0.1152	10	D5S1492	0.2329	55	D5S1457	0.5165	5	D5S2849
6	0.2916	40	D6S2439	0.6955	0	SE30	0.1062	35	D6S1959
7	0.0043	105	D7S821	0.0745	5	D7S3056	0.0222	70	D7S1818
8	0.2114	140	D8S1128	0.6149	10	D8S277	0.2432	130	D8S592–D8S1179
9	0.5513	65	D9S301	2.8406	80	D9S922	0.7469	65	D9S301
10	0.0872	155	D10S1213–D10S1248	1.3863	70	D10S1208–D10S1227	0.2481	155	D10S1213–D10S1248
11	2.4275	88	D11S2002	0.2656	105	D11S1986	2.0118	92	D11S2002–D11S2000
12	0.4772	40	D12S373–D12S1042	0.0546	95	D12S1064	0.3119	45	D12S1042
13	1.0041	99	D13S173–D13S285	0.0495	65	D13S317	1.2226	96	D13S173
14	0.2805	125	D14S1426	0.462	40	D14S599	0.1118	30	D14S608–D14S297
15	0.1194	0	D15S817	0.2333	45	D15S659	0.1591	0	D15S817
16	0	130	–	0.0231	70	D16S3253	0.0021	100	D16S516
17	1.8038*	60*	D17S1299*	0.8194	113	D17S784	2.0453	60	D17S1299
18	0.0371	105	ATA82B02	0.0005	0	GATA178F11	0.3993	105	ATA82B02
19	0.6571	70	D19S178	1.8181	66	D19S178	0.6371	80	D19S246
20	0.0759	55	D20S478	0.0632	65	D20S481	0.0983	55	D20S478
21	1.0558	3	D21S1432	0	55	–	1.1175	3	D21S1432
22	0.1185	0	D22S420	1.3386	0	D22S420	0.086	0	D22S420

* The LOD score of fasting serum insulin concentrations and markers on chromosome 17 only reflect suggestive linkage after adjustment for BMI (multipoint LOD 2.2 at ~66 cM from pter)

A genome scan was done to identify chromosomal regions that might contain genes influencing these traits and maximum multipoint LOD scores for insulin traits by chromosome with the associated marker and map location (Table 3). The regions with suggestive linkage (LOD >1.9) are highlighted. The results of the whole-genome scan for the traits fasting insulin, 2-h insulin and HOMA- (Fig. 1) multipoint linkage analyses on chromosome 11, 17 and 9 are shown (Fig. 2A–C).

On *chromosome 11*, suggestive evidence was found for linkage to fasting insulin concentrations at about 88 cM from pter (multipoint LOD 2.43, two-point LOD 2.04 at marker D11S2002 at 85.48 cM). When fasting insulin concentrations were adjusted for BMI, the LOD score dropped from 2.4 to 1.2 and the peak shifted about 10 cM, to about 101 cM. HOMA-IR showed a peak LOD in the same region (LOD 2.0 at 92 cM), with a similar decline when adjusted for BMI (Fig. 2A).

The 2-h insulin concentrations had no LOD score exceeding 0.4 on this chromosome.

On *chromosome 17* possible evidence was observed for linkage of fasting plasma insulin at about 60 cM from pter (multipoint LOD 1.8, two-point LOD 1.3 at marker D17S974 (GATA8c04)). When adjusted for BMI the multipoint LOD score became more suggestive, increasing to 2.2 while the peak shifted to 66 cM. In the same region we found evidence for link-

age to HOMA-IR values (multipoint LOD 2.0 at ~60 cM from pter) while after adjustment for BMI the LOD score dropped to 1.8 at 66 cM. Plasma 2-h insulin did not show substantial evidence for linkage to chromosome 17.

Two apparent linkage peaks for fasting insulin and HOMA-IR are seen on chromosome 17. The first peak at about 22 cM from pter with a multipoint LOD score of 1.4 (fasting insulin) and 1.2 (HOMA-IR), and the second peak at about 60 from pter for fasting insulin a two-point LOD 1.3 at marker D17S974 (GATA8c04) and with a multipoint LOD score of 1.8, and for HOMA-IR a multipoint LOD score of 2.0 [two-point LOD 1.5 at marker D17S974 (GATA8c04)], respectively. Although adjusting for BMI did not change the results of the highest peak, it rearranged the general shape of the graph to one peak at about 65 cM with a multipoint LOD score of 2.2 for fasting insulin (Fig. 2B).

On *chromosome 9*, 2-h plasma insulin showed a multipoint peak LOD score of 2.8 at 80 cM pter while the BMI adjusted values at the same location did not exceed a LOD of 0.35. The maximum LOD score of fasting insulin and HOMA-IR on chromosome 9 were 0.55 and 0.74, both at about 65 cM from pter.

On *chromosome 19* the multipoint LOD score for 2-h insulin was 1.8 at about 66 cM pter (two-point LOD 2.1), and showed minimal change after BMI adjustment. The maximum LOD score of fasting insulin

Genome-wide scan for loci linked to fasting insulin concentrations, 2h insulin concentrations and levels of HOMA-IR.

Fig. 1. Genome-wide scan for loci linked to fasting plasma insulin concentrations, 2-h plasma insulin concentrations and values of HOMA-IR

and HOMA-IR on chromosome 19 were 0.66 and 0.63, at about 65 cM and about 80 cM from pter, respectively (results not shown).

Other potentially interesting regions in which we found a multipoint LOD score exceeding 1.0, but less than 2.0 (Table 3) were on chromosome 1 at about 210–230 cM from pter, on chromosome 3 at about 205–220 cM from pter, on chromosome 10 at about 60–80 cM from pter, on chromosome 13 at about 90–100 cM from pter, on chromosome 21 at the p-terminal 0–5 cM, and on chromosome 22 at the p-terminal at 0–10 cM.

One thousand simulations of an unlinked marker to a simulated trait mimicking fasting insulin concentrations produced a median two-point LOD score of 0. In 1.9% of simulations a LOD score greater than 1.0 was obtained (mean 1.34, std.dev. 0.33), of which 0.1% had a LOD score greater than 2.0 (LOD score 2.05). No LOD score greater than 3.0 was found. For 2-h plasma insulin the simulations yielded a median two-point LOD score of 0.0001; 1.5% of simulations had a LOD score greater than 1.0 (mean 1.36, std.dev. 0.31), and 0.1% had a LOD score greater than 2.0 (LOD score 2.01). No LOD score greater than 3.0 was found.

Discussion

Our results suggest the existence of at least two genomic susceptibility loci influencing fasting plasma insulin and two loci influencing the 2-h insulin concentrations in a primarily Caucasian population sample. The results of fasting plasma insulin and insulin resistance estimated by HOMA-IR values were comparable and might reflect effects of the same gene(s). We found the strongest evidence for linkage with fasting plasma insulin and IR at about 88 to 92 cM from pter on chromosome 11. For 2-h plasma insulin the strongest evidence for linkage on chromosome 9 at about 80 cM from pter. These LOD scores decreased dramatically when the traits were adjusted for BMI. This effect of BMI adjustment suggests that the observed linkage between specific genomic loci and insulin traits is mediated in part by overall obesity, as reflected by BMI. This is consistent with studies in diverse populations showing that baseline weight or weight gain over many years increases the risk of incident Type 2 diabetes by 30% to 400% [20, 21, 22, 23,

24]. It remains uncertain how adipose tissue itself directly augments diabetes risk. Obesity could cause diabetes by inducing IR in the liver and skeletal muscle [25] or by impairing beta-cell function [26], possibly mediated through excessive adipocyte release of free fatty acids, tumour necrosis factor- α or other inflammatory cytokines or energy-related hormones. [27, 28, 29, 30, 31]. By these mechanisms obesity could represent a physiological “environmental” risk factor for Type 2 diabetes, such that BMI adjustment of genetic models could diminish the mediating effect of overall obesity, uncovering the marginal effects of other diabetes-related traits such as hyperinsulinaemia. Thus, obesity could confer a gene-“environment” interactive effect on risk for Type 2 diabetes. In contrast, obesity itself has a genetic basis, [32, 33] and some data suggest that specific loci linked to obesity traits could have pleiotropic effects, as one gene can have multiple, consequences that are seemingly unrelated. In that case the same gene influencing obesity could also affect plasma insulin and susceptibility to Type 2 diabetes [34, 34, 35, 36, 37]. From this perspective adjustment of linkage analyses for BMI could inappropriately obscure evidence for genes that influence other related Type 2 diabetes traits.

On chromosome 11q the evidence for suggestive linkage drops substantially for both traits after BMI adjustment. Several independent studies have reported linkage of BMI to the same area on chromosome 11 [34, 38]. However, in our case, it is unclear whether obesity leads to insulin resistance, or whether hyperinsulinaemia leads to obesity. Intriguingly, the *iddm4* locus for Type 1 diabetes also maps to this region [39], suggesting that a gene in this region could both account for insulin insufficiency in both Type 1 and Type 2 diabetes.

We found similar patterns for 2-h plasma insulin. After adjusting for BMI the evidence for linkage on chromosome 9 (~80 cM, LOD 2.8) disappeared completely (LOD=0.35) while the results on chromosome 19 seemed to be completely indifferent to BMI adjustment. Although high plasma insulin and IR are pre-diabetic phenotypes, and most likely have a less complicated genetic background than the diabetes as a whole, it is clear from these and other genome scans that BMI plays an unpredictable and poorly-defined role in the expression of some, but not all Type 2 diabetes susceptibility genes. Given the polygenic nature of obesity and Type 2 diabetes it is to be expected that each trait will reflect distinct gene-environment interactions. The regions on chromosomes 11 and chromosome 9 could represent pleiotropic loci, influencing both the insulin traits and BMI. The regions on chromosomes 17 and 19 seem to represent loci more specific to the insulin traits in response to other environmental cues. For example, the peak on chromosome 17 contains the gene for gastric-inhibitory peptide, a hormone that stimulates insulin secretion in response to oral glucose [40].

◀ **Fig. 2.** **A** Multipoint linkage analysis of markers on chromosome 11 with serum concentrations of fasting insulin (adjusted and not adjusted for BMI) and values of HOMA-IR. **B** Multipoint linkage analysis of markers on chromosome 17 with serum concentrations of fasting insulin (adjusted and not adjusted for BMI) and values of HOMA-IR. **C** Multipoint linkage analysis of markers on chromosome 9 with serum concentrations of fasting insulin, 2-h insulin concentrations and values of HOMA-IR

Several other studies have provided similar data on genomic associations with Type 2 diabetes traits. A genome scan on abdominal obesity-metabolic syndrome-related phenotypes in 507 Caucasian families, including plasma insulin concentrations reported two regions of interest, one on chromosome 3 and the other on chromosome 17p12 [37]. The trait linked to the chromosome 17 region was plasma leptin concentrations and the region was around 38 cM from pter. Interestingly, this seems to correspond with the small peak on our chromosome 17 result of fasting plasma insulin and HOMA-IR (Fig. 2B) that disappears completely when we adjust the traits for BMI.

In a genome scan in non-diabetic Pima Indians [4] a region on chromosome 11 was reported to be linked fasting plasma insulin. This region was the same as we report, but their results lost statistical significance in the multipoint analysis, and the LOD score dropped to 0.25. In another genome scan of Pima Indians, this time including diabetic subjects, mild linkage of Type 2 diabetes to a region on chromosome 11 was reported, LOD 1.7 (between D11S4464 and D11S912; 123 and 131 cM respectively on the Marshfield map) [34]. Although this peak is not exactly at the same location as ours (~88–102 cM from pter), the distance is about 35 cM and perhaps distant enough to be considered a separate finding.

In a genome scan in 719 Finnish sib-pairs with Type 2 diabetes an MLS of 1.75 was found in exactly the same region on chromosome 11 (84 cM) [41]. In a QTL analysis in the same population, however, this region has not been replicated [6]. Other reported findings of linkage between insulin traits and loci on chromosome 11 mostly refer to 11p, like *sur1* at 11p15.1 at 20–26 cM pter and refer clearly to a different chromosomal region [42].

In an earlier genome scan in non-diabetic Pima Indians [4], several regions linked to 2-h plasma insulin are mentioned, the highest multipoint peak being at 9q21 (LOD 2.16 at 99 cM) which falls in the same region we found suggestive evidence for linkage with 2-h insulin concentrations (LOD 2.4 at 84 cM). In a genome-wide scan in 102 Chinese families, for Type 2 diabetes, affected sib-pair analysis revealed two markers on chromosome 9 that showed suggestive evidence, D9S171 (43 cM) and D9S175 (70 cM) with an NPL of 3.3 and 2.9, (p values 0.00012 and 0.00045) respectively [43]. As all these linkage signals give LOD scores below genome-wide significance, replication in other independent cohorts or populations are needed. In this light our findings on chromosomes 11 and 9 are important and strengthen the evidence shown in previously published studies.

Although none of our results exceed a LOD score of 3.3, the analysis of simulated unlinked markers indicated that our findings are unlikely to be spurious, false positive peaks. For each trait there was a less than 2 in 1000 chance of finding a peak with a two-point LOD of

1.0 or higher. In a genome scan of 401 markers fewer than one LOD score over 1 would be expected to be due to type I error, while the expected rate of a LOD score over 2 due to type I error would be close to 0.

In summary, we found suggestive evidence for linkage of some regions to several insulin traits implicated in the pathogenesis of Type 2 diabetes on chromosomes 9, 11 and 17. Substantial attenuation of linkage signals when plasma insulin concentrations were adjusted for BMI suggest either pleiotropy for both insulinaemia and obesity at these loci, or that overall obesity directly mediates associations of these loci with plasma insulin concentrations. Further work is required to test associations of candidate genes in these regions with occurrence of Type 2 diabetes and/or obesity, as well as to map other likely diabetes genes to these regions.

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