Genotype-by-sex interaction in the aetiology of type 2 diabetes mellitus: support for sex-specific quantitative trait loci in Hypertension Genetic Epidemiology Network participants

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Abstract

Aims/hypothesis While there are sex-related differences in both the prevalence of type 2 diabetes mellitus and disease risk factors, there is only limited research on sex-specific influences on type 2 diabetes aetiology within the same study population. Thus, we assessed genotype-by-sex interaction using a liability threshold model in an attempt to localise sex-specific type 2 diabetes quantitative trait loci (QTLs).

Subjects, materials and methods Hypertensive siblings and their offspring and/or parents in the Hypertension Genetic Epidemiology Network of the Family Blood Pressure Program were recruited from five field centres. The diabetic phenotype was adjusted for race, study centre, age and non-

linear age effects. In total, 567 diabetic individuals were identified in 385 families. Variance component linkage analyses in the combined sample and stratified by sex and race were performed (SOLAR program) using race-specific marker allele frequencies derived from a random sample of participants at each centre.

Results We observed a QTL-specific genotype-by-sex interaction ($p=0.009$) on chromosome 17 at 31 cM, with females displaying a robust adjusted logarithm of odds (LOD) of 3.0 compared with 0.2 in males and 1.3 in the combined sample. Three additional regions demonstrating suggestive evidence for linkage were detected: chromosomes 2 and 5 in the female sample and chromosome 22 (adjusted LOD=1.9) in the combined sample.

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Conclusions/interpretation These findings suggest that multiple genes may regulate susceptibility to type 2 diabetes, demonstrating the importance of considering the interaction of genes and environment in the aetiology of common complex traits.

Keywords Genome scan · Genotype-by-sex interaction · Type 2 diabetes mellitus

Abbreviations

ARP	affected relative pair
cM	centimorgan
ESM	Electronic Supplementary Material
GRR	Graphical Representation of Relationships
HyperGEN	Hypertension Genetic Epidemiology Network (of the Family Blood Pressure Program)
IBD	identical-by-descent
LOD	logarithm of odds
MAP2K4	mitogen-activated protein kinase 4 gene
NCOR1	nuclear receptor corepressor 1 gene
PPARG	peroxisome proliferator-activated receptor γ
QTL	quantitative trait locus

Introduction

Susceptibility to type 2 diabetes mellitus is determined by multiple genetic and environmental factors and interactions between the two. While there are sex-related differences in both the prevalence of type 2 diabetes and disease risk factors, research on complex interactions by sex in the pathogenesis of type 2 diabetes is limited [1, 2]. Whether sex-specific genetic effects contribute to differences in type 2 diabetes status also warrants further investigation [3–5].

Modelling the true complexity of type 2 diabetes may advance our understanding of the aetiology of this major disease. For example, the identification of genotype-by-sex interactions could improve the localisation of type 2 diabetes quantitative trait loci (QTLs) and increase the support for linkage. Such findings may also help explain why females have a higher prevalence of type 2 diabetes in some populations [6–8], but not others [9].

We previously detected QTLs for type 2 diabetes status on chromosomes 22, 8 and 17 [10]. Since that previous study did not consider complex patterns of interaction by sex, and given that such patterning has been suggested in the literature reviewed above, we examined the evidence for genotype-by-sex interaction in the liability to type 2 diabetes by reanalysing a sample of siblings and/or their first-degree relatives from the HyperGEN (Hypertension Genetic Epidemiology Network of the Family Blood Pressure Program) cohort. The HyperGEN population may

be ideal for further investigation of type 2 diabetes candidate genes considering that genetic heterogeneity (e.g. alleles at multiple loci conferring susceptibility to the trait of interest) complicates the identification of genes influencing common chronic diseases like type 2 diabetes by reducing study power. Thus, the heterogeneous nature of the disease indicates that homogeneous subgroups such as HyperGEN may represent the best strategy for susceptibility gene identification [11].

Subjects, materials and methods

Participants

This study examined participants in HyperGEN. Informed consent was obtained from all participants and this project was approved by the Institutional Review Boards of all participating institutions. HyperGEN methods [12] and eligibility criteria for this analysis [10] have been reported previously. Briefly, hypertensive siblings and their offspring and/or parents were recruited from five field centres located in the States of Massachusetts, North Carolina, Minnesota, Utah and Alabama. Sibship ascertainment criteria required sibships of two or more siblings with hypertension (defined as blood pressure $\geq 140/90$ mmHg or self-reported use of antihypertensive medications) in which age at hypertension diagnosis did not exceed 60 years.

Phenotyping

Participants were classified as having a diabetic phenotype if they reported using hypoglycaemic medications and/or their fasting serum glucose concentrations exceeded 7 mmol/l. Individuals reporting an age at diagnosis < 30 years were excluded from analyses in an attempt to remove type 1 diabetic persons. Serum glucose concentrations were measured with the Elan Glucose Reagent [13]. Briefly, the hexokinase phosphorylates glucose with ATP, producing glucose-6-phosphate. The product is then oxidised by glucose-6-phosphate dehydrogenase to form 6-phosphogluconate, with concurrent reduction of NAD to NADH. The ensuing increase in absorbance at 340 nm is directly related to the concentration of glucose in the sample.

Genotyping

Genotyping was performed by the NHLBI Mammalian Genotyping Service (Marshfield, WI, USA) (<http://research.marshfieldclinic.org/genetics/>, last accessed March, 2006). Cooperative Human Linkage Centre screening set eight was used, which included 387 microsatellite markers spaced

(approximately 10 centimorgan [cM] distance) throughout the genome. Average marker heterozygosity was 77.7%.

Quality control in several aspects on markers was performed. Relationship status among siblings was evaluated applying ASPEX, a likelihood-based method [14]. Mapmaker/Sibs [15] and PedCheck [16] were utilised to find and eliminate any Mendelian marker inconsistencies. Additionally, we have now completed advanced pedigree cleaning using the Graphical Representation of Relationships (GRR) program [17], where relationship mis-specifications are detected by pair-wise identity-by-state allele sharing. Using GRR, we determined if a reported pair-wise relationship was consistent with genotype data, and if not, we identified the most likely relationship given the data. We used the University of California Santa Cruz (<http://genome.ucsc.edu>, last accessed March, 2006) and the Wellcome Trust Sanger Institute (<http://www.sanger.ac.uk/>, last accessed March, 2006) websites to determine the cytogenetic location of markers and to search for candidate genes.

A loss of genotype data summary in the phase I HyperGEN sample is as follows: genotyping failure (4.3%), family structure corrections and removal of monozygotic twin marker data (1.16%), genotyping error and removal of marker data due to inconsistent allele calling within families (0.71%), and inconsistent allele calling (1.42%). Thus, the total loss of genotype data due to genotyping problems, genotyping errors and the cleaning process was 7.59%

Statistical genetic methods

Linkage of type 2 diabetes status was examined using a variance component model in the combined sample and in each sex separately. Genome-wide logarithm of odds (LOD) scores were estimated using the program package SOLAR [18]. This approach is applicable to dichotomous traits under the assumption that an individual is classified as affected if an underlying genetically influenced liability, which is presumed to have a multivariate normal distribution, exceeds a given threshold [19].

Linkage to type 2 diabetes status was also tested using the variance component linkage model extended to include genotype-by-sex interaction at a QTL [20–22]. The expected genetic covariance between male and female relative pairs (i, j) is: $\text{Cov}(g_{iM}, g_{jF}) = 2\phi_{ij}\rho_{g(M, F)}\sigma_{gM}\sigma_{gF} + \pi_{ij}\sigma_{qM}\sigma_{qF}$, where the subscripts M and F refer to male and female, ϕ is the coefficient of kinship between two individuals, $\rho_{g(M, F)}$ is the additive genetic correlation between the expressions of the trait in the two sexes, σ_{gM} and σ_{gF} are the genetic SDs for males and females, π_{ij} is the probability that individuals i and j are identical-by-descent (IBD) at a QTL linked to a genetic marker locus, and σ_{qM} and σ_{qF} are the marker-specific genetic SDs for males and females, respectively.

The absence of additive genotype-by-sex interaction (the null hypothesis) corresponds to the genetic correlation between a male and female relative pair equalling one ($\rho_{g(M, F)}=1.0$). Male and female genetic SDs should also be equivalent under the null hypothesis ($\sigma_{gM}=\sigma_{gF}$). Each hypothesis is evaluated separately by comparing the likelihood of a model with one parameter constrained to the likelihood of the full model with all parameters estimated. The test statistic in analyses examining genetic correlation is a mixture of chi-square with one degree of freedom and a point mass at zero, as the parameter is constrained to a boundary. In the case of the additive genetic variances, the test statistic is a one degree of freedom chi-square, as no boundary is involved. Although there is a compound null hypothesis, $\rho_g=1$ and $\sigma_{gM}=\sigma_{gF}$, we tested for different magnitudes of additive genetic variance and different sources of additive genetic variance individually in separate likelihood ratio tests that each have a single parameter constrained. For example, when testing for $\rho_g<1$, separate additive genetic variances for males and females are estimated and when testing for different additive genetic variances ρ_g is allowed to float. The null hypothesis of no genotype-by-sex interaction is rejected if either of these tests is significant at $\alpha=0.05$.

In addition, we added the marker-specific parameters to the model (σ_{qM}, σ_{qF}) and tested for QTL-specific genotype-by-sex interaction. Marker-specific genotype-by-sex interaction is indicated when the model likelihood in which the male and female marker-specific SDs are constrained to be equal ($\sigma_{qM}=\sigma_{qF}$) (i.e. the null hypothesis) is compared with the likelihood of a general model, in which all additive and QTL-specific parameters are estimated. All genotype-by-sex interactions were assessed using likelihood-ratio tests ($\alpha=0.05$) that compare the likelihood of a model that includes a genotype-by-sex interaction parameter against a model that restricts genotype-by-sex interaction [23–25].

Although linkage analysis is robust to ascertainment, accounting for sampling and ascertainment can increase the power to detect a QTL [26] and improves generalisability of linkage findings to the general population [27]. Because the HyperGEN recruitment protocol required sibships of two or more hypertensive persons, an exact ascertainment correction for proband status was unavailable. Therefore, we chose one randomly selected proband from each family, as Comuzzie and Williams [26] demonstrated that this correction is easily implemented and appears highly effective in improving the estimation of population parameters and decreasing type II error.

Linkage findings were evaluated by examining the empirical distribution of the LOD scores, under the assumption of multivariate normality, using 10,000 replicates and simulation methods incorporated into SOLAR [18]. The empirical distribution of the simulated LOD

scores was used to assign percentiles to each replicate and estimate an expected test statistic. SOLAR then produced a constant to correct for the non-normality of the phenotype by regressing the expected LOD scores on the simulated LOD scores, which were then used to determine the adjusted genome-wide LOD scores (adjusted LOD score = observed LOD score $\times$ correction constant) [28].

The variance component approach required IBD matrix estimates. The small family sizes of HyperGEN participants permitted us to calculate exact conditional probabilities employing the Lander–Green algorithm as implemented in MERLIN [29]. Allele frequencies from a random sample of participants at each field centre were calculated separately in the African-American and Caucasian groups. The IBD probabilities computed by MERLIN were then combined into a single set of multipoint IBD files in the SOLAR format using the program Mer2sol (<http://taxa.epi.umn.edu/mer2sol/>, last accessed March, 2006) developed by Michael B. Miller at the University of Minnesota.

Application to HyperGEN data

The diabetic phenotype was adjusted for race, centre, age and non-linear age effects (see Table 1 of the Electronic Supplementary Material [ESM]). While we also examined the influence of adiposity by adjusting for percentage body fat, the main conclusions were unaltered. No additional covariate adjustments were made, since we investigated prevalent type 2 diabetes status and did not know the true covariate values at disease onset.

Results

The characteristics of covariates in the combined sample and stratified by sex are shown in Table 1 and the distribution of relative pairs in the combined sample and

Table 2 Distribution of relative pairs among 3,153 HyperGEN participants in the combined sample and stratified by sex for all participants and affected individuals only

Relative pair	Combined sample	Females	Males
All participants ($n=3,153$)			
Total	3,947	1,380	813
Parent–offspring	652	251	112
Siblings	1,931	682	417
Avuncular	823	252	172
Half-siblings	218	90	40
First cousins	194	45	58
Other	129	60	14
Affected individuals only ($n=567$)			
Total	155	69	30
Parent–offspring	27	16	4
Siblings	112	46	22
Avuncular	4	2	1
Half-siblings	12	5	3
First cousins	0	0	0
Other	0	0	0

by sex is presented in Table 2. Eighty percent of the 567 ($n=412$) participants classified as diabetic reported hypoglycaemic medication use. Sibling relative pairs were most often represented due to the study design. Five hundred African-American and 419 Caucasian families were available for analysis, of which 260 (52%) African-American and 159 (38%) Caucasian families had at least one diabetic member. One hundred and 57 families had at least one male diabetic member, with two families having three male diabetic members and 24 families having two diabetic members. Female diabetic members were more prevalent, as 274 families had at least one diabetic member, with one family having four diabetic members, seven families having three female diabetic members, and 42 families having two female diabetic members.

Table 1 Covariate characteristics in the combined sample and by sex for HyperGEN participants

	Complete sample	Females	Males
Total	3,153	1,883	1,270
Diabetic, n (%)	567 (18.0)	362 (19.2)	205 (16.1)
Hypertensive, n (%)	2,417 (76.7)	1,463 (78.9)	954 (75.6)
Mean age, years (SD)	52.6 (13.6)	52.3 (13.6)	53.1 (13.7)
Race/centre, n (%)			
Caucasians, NC	185 (5.9)	129 (6.8)	56 (4.4)
Caucasians, MN	408 (12.9)	199 (10.6)	209 (16.5)
Caucasians, MA	435 (13.8)	228 (12.1)	207 (16.3)
Caucasians, UT	517 (16.4)	260 (13.8)	257 (20.2)
African-Americans, NC	396 (12.6)	278 (14.8)	118 (9.3)
African-Americans, AL	1,212 (38.4)	789 (41.9)	423 (33.0)

AL Alabama, MA Massachusetts, MN Minnesota, NC North Carolina, UT Utah

The multipoint adjusted genome-wide LOD scores for the combined sample and stratified by sex for all peaks with an adjusted LOD score >1.8 (suggestive evidence of linkage) [30] are given in Table 3. We detected a QTL influencing liability to type 2 diabetes status in females with a corresponding adjusted LOD score of 3.0 on chromosome 17p12 at map location 31 cM (nearest marker AFM290VC9) (Fig. 1). This signal was considerably higher than the adjusted LOD scores observed in the complete (adjusted LOD=1.3) or male (adjusted LOD=0.2) samples. Three additional regions demonstrating suggestive evidence for linkage were also detected. Two were identified on chromosomes 2 and 5 in the female sample (adjusted LOD=1.8 and 1.9, respectively) and the third was detected on chromosome 22 (adjusted LOD=1.9) in the combined sample.

In addition, we formally assessed genotype-by-sex interaction. We found suggestive evidence of distinct additive genetic effects on type 2 diabetes status in males and females ($\rho_g=0.58\pm0.24$, $p=0.051$). We also assessed QTL-specific genotype-by-sex interaction at our maximum linkage peak on chromosome 17. Sizeable QTL-specific genotype-by-sex differences ($p=0.009$) were detected on chromosome 17 at map location 31 cM ($\sigma_{qM}=0.13\pm1.00$, $\sigma_{qF}=1.23\pm0.92$).

To assess the contribution of singletons, unaffected relative pairs, and affected relative pairs (ARPs) to the linkage signal on chromosome 17, we also performed multipoint model-free ARP linkage analysis as implemented in MERLIN [31]. We used race-specific allele frequencies and examined the false-positive proportion in MERLIN using the ‘-simulate’ command, in which 10,000 marker datasets were constructed under the null hypothesis of no linkage to type 2 diabetes. Fifty-seven African-American and 45 Caucasian families contributed to the analysis, in which an LOD score of 2.2 ($p=0.0005$) at 31 cM was observed, thus demonstrating a substantial contribution of ARPs to the evidence for linkage. Power constraints did not facilitate ARP linkage by sex.

Table 3 Estimated adjusted LOD scores suggestive of linkage (LOD ≥ 1.8 , Rao and Gu [30]) in the combined sample and by sex from multipoint quantitative trait linkage analyses of derived type 2 diabetes status in 3,153 HyperGEN participants

Sample	Chromosome	cM	LOD	Cytogenic location
Females	2	167	1.8	2q24.2
Females	5	143	1.9	5q31.1
Females	17	31	3.0	17p12
Combined	22	28	1.9	22q12.1

Fig. 1 Estimated robust adjusted LOD functions overall and by sex obtained from multipoint quantitative trait linkage analyses of type 2 diabetes status for chromosome 17 (green, females; red, males; blue, combined sample)

Discussion

We previously reported linkage of liability to type 2 diabetes status on chromosome 22q12.1 (adjusted LOD=1.9), 8q11.23 (adjusted LOD=1.6), and 17q11.2 (adjusted LOD=1.6) [10]. In this study, we extended our previous analyses to refine the localisation of these putative QTLs and further describe the genetic architecture of type 2 diabetes. Specifically, we modelled genotype-by-sex interactions, as previous work suggested strong sex effects. Indeed, modelling the true complexity of type 2 diabetes by incorporating genotype-by-sex effects may aid in the resolution of previously identified suggestive linkage peaks and improve QTL localisation by increasing the power to detect linkage [32].

We detected a QTL influencing liability to type 2 diabetes status in the female sample with a corresponding adjusted LOD score of 3.0 on chromosome 17p12. This signal was considerably higher than the adjusted LOD scores observed in the male (adjusted LOD=0.2) and combined (adjusted LOD=1.3) samples.

Significant QTL-specific genotype-by-sex interaction was detected on chromosome 17 at 31 cM. Interestingly, the incorporation of sex-specific genetic effects shifted the maximum linkage peak 20 cM towards the p terminus, outside the LOD drop support interval for the QTL detected in the combined sample. It is unclear which factors are influencing this shift; possibilities include low information content of the markers between the linkage peaks or two QTLs. However, an oligogenic linkage analysis did not support the presence of a second QTL on chromosome 17

(results not shown). Our future work to identify the specific functional variants influencing liability to type 2 diabetes may consider a much larger region of chromosome 17 (27 Mb) than previously indicated (17 cM, 6 Mb) [10], as we were unable to distinguish between poor localisation or two underlying QTLs.

To assess how robust our findings were to the partial hypertensive ascertainment correction, we also performed an ascertainment correction that accounted for the high prevalence of type 2 diabetes in this population, as families were recruited for hypertension, a trait related to type 2 diabetes. Following the approach of Cripe et al. [33], we constrained the sex-specific prevalence of type 2 diabetes using a range of population estimates (10–30%) [34] in the polygenic model, as previous simulation analyses have demonstrated the effectiveness of this approach (J. Blangero, Southwest Foundation for Biomedical Research, San Antonio, TX, USA, personal communication, 2003). Our results indicated that the LOD scores were stable across approaches to the ascertainment correction, producing LOD scores at the same location and of similar magnitude (± 1 LOD unit, results not shown).

Differences in the genetic expression of type 2 diabetes or differences in genes contributing to the sex-related variation in disease susceptibility genes are plausible and supported by several lines of evidence. Multiple studies have suggested sexual dimorphism in type 2 diabetes rates, such as the German National Health Interview and Examination Survey [8] and the MONICA Augsburg Cohort Study [3]. Ling et al. [35] also demonstrated that insulin-stimulated peroxisome proliferator-activated receptor γ (PPARG) coactivator 1 α expression, a gene implicated in the activation of multiple metabolic pathways in skeletal muscle, adipose tissue, liver and pancreas, was inversely related to sex (reduced expression in females, $p < 0.0001$) in 98 non-diabetic monozygotic and dizygotic twin pairs.

Furthermore, Hegele et al. [36] demonstrated odds ratio modification by sex ($p = 0.04$) of *PPARG*, a gene modulating insulin sensitivity and adipocyte differentiation, in adult Oji-Cree Canadian Indians who were homozygous for the hepatocyte nuclear factor-1 α G319 allele. Whereas females with at least one copy of *PPARG* A12 had an odds of type 2 diabetes that was 2.80 (95% CI 1.61–4.89) times the odds of type 2 diabetes among P12 homozygotes, the estimated odds ratio for males was 0.82 (95% CI 0.36–1.87) for the same contrast.

While we interpret the finding of significant linkages in one sex but not in the other as likely to represent sex-specific genetic effects, another and perhaps more simple explanation may be a lack of power to detect linkage in males, as 59.7% of the sample were female. However, by formally modelling a genotype-by-sex interaction, we were able to include the

2,193 pairs of relatives discordant for sex which were not included in our linkage analysis stratified by sex, thus improving our ability to detect genetic effects. Females displayed a larger QTL-specific genetic SD (1.23 ± 0.92) in comparison with males (0.13 ± 1.00), yet large SEs were noted. In addition, our simulation analyses suggested that the non-adjusted LOD scores were slightly overestimated for both males (LOD correction constant=0.94) and females (LOD correction constant=0.91).

The 1-LOD drop support interval for the signal on 17p12 overlaps signals from numerous genome-wide scans related to obesity measures [37–39] and one for type 2 diabetes [40]. Lindgren et al. [40] detected suggestive evidence of linkage to type 2 diabetes in 58 multiplex Finnish families on 17p12–p11 (multipoint LOD=1.9). The 17p12 locus was also implicated in several studies using variance components methods examining obesity phenotypes. Kissebah et al. [38] detected linkage to plasma leptin (LOD=5.0) in 2,209 individuals distributed over 507 nuclear Caucasian families, whereas Comuzzie et al. [39] identified linkage to adiponectin in 1,100 adults of predominantly northern European ancestry within 170 families (LOD=1.7). Linkage to BMI (LOD=2.47) was also observed in 753 Caucasian members of nuclear families identified through young to middle-aged probands with elevated blood pressure [37].

Several candidate genes underlie the 1-LOD unit drop support interval on chromosome 17 (17 cM, 6 Mb) including those for mitogen-activated protein kinase 4 (*MAP2K4*) and nuclear receptor corepressor 1 (*NCOR1*). Of note are also the ATP citrate lyase, gastrin, insulin-like growth factor-binding protein 4, and transcription factor 2, hepatic, genes, if we considered the larger 27-cM interval.

Lee et al. [41] demonstrated moderate differential *MAP2K4* expression in the skeletal muscle of six pairs of insulin-sensitive and insulin-resistant Pima Indians matched for percent body fat. *NCOR1* has been shown to interact with all three forms of PPAR [42], well known candidate genes for type 2 diabetes. PPAR is a short-lived protein that modulates expression of several genes implicated in lipid and glucose metabolism [43]. Overexpression of *NCOR1* results in a sizeable decrease of PPAR transcriptional activity. PPAR overexpression has implications in insulin resistance, as Bernal-Mizrachi et al. [44] showed that PPAR expression evoked through dexamethasone treatment in PPAR wild-type, but not PPAR-null, mice induced gluconeogenic gene expression.

No other genome-wide significant or suggestive QTLs were detected in the sample stratified by sex, although we detected suggestive evidence of linkage on chromosomes 2 and 5 in females. Further adjustment with percent body fat also recovered our previously reported QTL on 8q24.11 (adjusted LOD=2.0). These suggestive linkage signals have been de-

tected (based on varying criteria) in other genome screens of type 2 diabetes phenotypes, for example on 22q12.1 [45–47], 2q24.2 [48], 5q31.1 [49] and 8q24.11 [50].

This study may have been limited by our inability (1) to remove linear effects of additional factors influencing type 2 diabetes susceptibility, as we are investigating prevalent disease status, or (2) to stratify by other effect modifiers. We also lacked information on the age of type 2 diabetes onset for 99 participants we classified as diabetic, which may have introduced type 1 diabetic participants into our analysis. However, type 2 diabetes onset prior to age 21 was an exclusion criterion at the HyperGEN field centres during participant enrolment. We also excluded 44 individuals reporting an age at diagnosis <30 years, making it likely that nearly all diabetic persons were type 2, despite missing age at onset in our subsequent analysis.

We recognise that multiple testing is a substantial issue in the analyses of gene-environment interactions. We attempted to limit the impact of this problem by focusing extensive analyses only on those regions that demonstrated differences in evidence of linkage by sex. Although this strategy led to fewer statistical tests, it may have introduced some bias. Furthermore, while we interpret the finding of significant linkages in one sex but not the other as likely to represent sex-specific genetic effects, another and perhaps more simple explanation may be that there is sufficient power to detect linkage in only one sex. Clearly further independent research is needed to confirm and refine this work.

In conclusion, we observed significant linkage results for a QTL related to the diabetic phenotype on chromosome 17 in females. We also detected suggestive evidence of linkage on chromosomes 2 and 5 in females and 22 in the complete sample. Although we detected increased support for linkage at the chromosome 17 QTL, incorporating genotype-by-sex effects did not improve QTL localisation. These signals replicate regions previously linked to type 2 diabetes status and there are plausible candidate genes for the signal detected on chromosome 17, such as *MAP2K4* and *NCOR1*. These findings suggest that multiple genes may regulate susceptibility to type 2 diabetes and demonstrate the importance of considering the interaction of genes and environment in the aetiology of common complex traits.

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