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SHORT COMMUNICATION

A German genome-wide linkage scan for type 2 diabetes supports the existence of a metabolic syndrome locus on chromosome 1p36.13 and a type 2 diabetes locus on chromosome 16p12.2

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Abstract
Aims/hypothesis The aim was to identify type 2 diabetes susceptibility regions in 250 German families.
Subjects and methods We conducted a genome-wide linkage scan using 439 short tandem repeat polymorphisms at an average resolution of 7.76±3.80 cM (Marshfield). In an affected-only-design (affected sib pairs), we performed nonparametric multipoint linkage analyses. Conditional analyses were applied where linkage signals were found in the baseline analyses.
Results We identified two loci with nominal evidence for linkage on chromosomes 1p36.13 and 16p12.2 (D1S3669, 37.05 cM, logarithmic odds ratio [LOD]=1.49, p=0.004; D16S403, 43.89 cM, LOD=1.85, p=0.002). D16S403 crossed the empirically obtained threshold of genome-wide suggestive significance of LOD=1.51. Positive findings in those regions have been reported by the following other linkage studies on: (1) symptomatic/clinical gall bladder disease with type 2 diabetes in Mexican Americans from the San Antonio Family Diabetes/Gallbladder Study (LOD=3.7, D1S1597–D1S407, 29.93–33.75 cM); (2) body

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size-adiposity in another Mexican American population (D1S1597, LOD=2.53, 29.93 cM); (3) lipid abnormalities (LOD=3.1, D1S2826–D1S513, 41.92–60.01 cM); and (4) hypertension in Australian sib pairs (LOD=3.1, D1S2834–D1S2728, 31.02–33.75 cM); as well as (5) a meta-analysis of four European type 2 diabetes-related genome scans (LOD=1.09, D16S412, 42.81 cM). In linkage analyses conditional on evidence for linkage at D16S403 we identified a LOD increase (ΔLOD) of 1.55 (p=0.0075) at D17S2180. Similar conditioning on D17S2180 revealed evidence for interaction with D1S3669 (ΔLOD=1.67, p=0.0055), D16S403 (ΔLOD=1.48, p=0.0091) and another locus on chromosome 1 where several genome scans have reported evidence for linkage (~200 cM, ΔLOD=1.60, p=0.0066).
Conclusions/interpretation Our results and the findings of other studies are consistent with the presence of a locus for a complex metabolic syndrome on chromosome 1p36.13.

Keywords Clinical science · Conditional linkage analysis · Genetics of type 2 diabetes · Genomewide scan · Linkage studies · Metabolic syndrome · Susceptibility region

Abbreviations
LOD logarithmic odds ratio
Prop proportional to the evidence for linkage

Introduction

Whereas the identification of at least six genes for the rare autosomal–dominant forms of MODY was remarkably successful, only modest progress has been made for common type 2 diabetes. The first successful genome-wide scan in 330 affected Mexican American sib pairs with type 2 diabetes found linkage to chromosome 2q37 (D2S125–D2S140, logarithmic odds ratio [LOD]=4.15). Using an association approach, a haplo(geno)type consisting of three polymorphisms with functional impact was identified within the calpain-10 gene [1]. The study was successful because another interacting locus on chromosome 15—obtained by conditional analyses—was considered [2].

In Germany, type 2 diabetes shows increasing prevalence with 5–8 million people having some form of diabetes (prevalence: 6–10%). In an effort to identify causative genetic factors, we report here results of linkage studies in which we identified two type 2 diabetes loci. We elucidated potentially interacting regions by conditioning our sample on the positive linkage signals identified. Taken together, our results and the findings of other studies provide evidence for a complex metabolic syndrome locus on chromosome 1p36.13.

Subjects and methods

Subjects Patients were identified through the Department of Metabolic Disorders of the University Clinic Dresden and through diabetologists in private practice. Blood samples were used for DNA extraction and baseline laboratory parameters. Patients were all of German origin living in Saxony (south-eastern Germany). The ethics committee approved this study; informed consent was obtained from all subjects. Primary inclusion criteria were: (1) only affected subjects with type 2 diabetes according to WHO criteria; (2) at least two affected sibs per sibship; and (3) age at diagnosis ≥35 years. Patients were excluded if they had: (1) suspected MODY (at least three generations with autosomal–dominant diabetes; at least 2 years of treatment with diet and/or glucose-lowering drugs other than insulin; age at diagnosis ≤25 years); or (2) type 1 diabetes (atypical manifestation of type 2 diabetes, with antibody testing for islet cell antibodies, insulin, GAD64 being conducted in such cases and in 359 randomly selected patients). Parents were not available for our study. Clinical characteristics of the sample are summarised in Table 1.

Genotyping We screened 439 microsatellite markers with an average marker density of 7.76±3.80 cM (Marshfield), no single intermarker distance of >15 cM, an average heterozygosity of 0.765±0.068 and a call rate of ≥95%. Five markers in Hardy–Weinberg disequilibrium were

Table 1 Clinical characteristics of affected individuals included in the analysis

Parameter	Women	Men
Subject number	300	231
Height (m)	1.62±0.07	1.73±0.08
Weight (kg)	77.36±13.83	83.56±14.08
BMI (kg/m^2)	29.53±4.81	27.73±3.81
Blood pressure systolic/diastolic (mmHg)		
Systolic	147±20	143±18
Diastolic	82±10	82±10
Age at diagnosis (years)	51±13	47±12
Treatment (%)		
Diet	50.66	50.66
Oral hypoglycaemic agents	60.74	63.76
Insulin	45.19	47.16
HbA_{1c} (%)	7.21±1.22	7.54±1.87
Creatinine (μmol/l)	92.52±42.02	103.41±34.77
Uric acid (μmol/l)	282.29±96.37	298.15±97.91
Total cholesterol (mmol/l)	6.12±1.40	6.01±2.62
Total triacylglycerol (mmol/l)	2.53±1.96	2.35±1.70
HDL-cholesterol (mmol/l)	1.27±0.39	1.18±0.47
LDL-cholesterol (mmol/l)	3.74±1.10	3.57±1.63

Total number of affected individuals: 531. Data are means ± standard deviation (SD).

removed (AFMA312XG5, D3S2395, D7S820, D11S2632, D14S617). Mendelian/genotyping errors were identified by Pedcheck [3] and Merlin [4], and removed or retyped, respectively.

Genetic relationships among family members were checked by Graphical Relationship Representation [5]. Misclassification of half-siblings as full siblings (19 families with 22 half-sib pairs) was identified and resolved. Four affected sib pairs with identical genotypes of both siblings (either not previously reported as twins or possible sample duplication), four identical twins and four affected sib pairs where siblings previously reported to be related turned out to be unrelated to each other were removed. We analysed 250 families comprising 290 affected sib pairs and 22 half-sib pairs and corresponding to 531 affected non-founders (231 males, 300 females). The sample structure was as follows: 203 (28) families with two (three) affected siblings, 16 families with two affected individuals in a half-sib relationship and three families with two affected siblings and one affected half-sibling.

Linkage analyses, simulations All analyses were run under the graphical user interface easyLINKAGE (http://sourceforge.net/project/showfiles.php?group_id=124875). Marker allele frequencies were estimated from our sample. Nonparametric two-point linkage analysis was performed with Splink [6, 7], multipoint linkage analysis with Merlin using the score-pairs statistic. Accurate estimates of the genome-wide statistical significance of linkage results were obtained empirically by simulating 1,000 replicates derived from our dataset using Merlin and by performing the locus-counting approach [8]. Allegro [9] was used for conditional analyses. The evaluation of the significance of p values and the generation of family weights were performed as described recently [2].

Statistical power, simulation studies For details [see Electronic supplementary material (ESM)].

Results

Two-point nonparametric linkage analyses revealed six regions on chromosomes 1, 2, 3, 7, 11 and 16 with a LOD≥1. The strongest signal was obtained on chromosome 3 (LOD=2.87, nominal p=0.0003 at D3S3050) (ESM Table 1). Two of those loci reached a nonparametric multipoint LOD>1 (D1S3669, LOD=1.49, nominal p=0.004; D16S403, LOD=1.85, nominal p=0.002) (Fig. 1; ESM Table 2). The region around D16S403 crossed the level of genome-wide suggestive linkage (ESM Table 3).

Conditional analyses revealed no significant interaction between chromosomes 1 and 16 (ESM Table 4; ESM Fig. 1a,b, ESM Fig. 2a,b). However, when conditioning on D16S403, we identified an interaction with D17S2180 ($LOD_{baseline/prop}$=0.08/1.63, p=0.0075 for LOD increase [ΔLOD], where prop=proportional to the evidence for linkage; ESM Fig. 1c,d, ESM Fig. 2b). When conditioning on D17S2180, the interval D1S3669–D1S1622 showed a ΔLOD from 1.14 to 2.81 (p=0.0055, weight 0–1) and a region on chromosome 1 at ~200 cM also showed a significant ΔLOD (ΔLOD=1.60, p=0.0066; ESM Fig. 1e,f, ESM Fig. 2c). In addition, the LOD score at D16S403 increased from 1.85 to 3.33 (p=0.0091, weight prop).

Discussion

Our hypothesis is that the clinical heterogeneity of type 2 diabetes is related to underlying genetic heterogeneity. Thus we focused our studies on families with a more homogeneous form of diabetes transmission, using clinical and laboratory-based measurements to minimise contamination by other known subtypes of diabetes such as MODY or type 1 diabetes, and by recruiting patients from a defined geographical area with German ancestry.

In both the two- and multipoint linkage analyses, two loci on chromosomes 1 (37.05 cM, D1S3669) and 16 (43.89 cM, D16S403) showed nominal and suggestive evidence for linkage, respectively. Close to our finding on chromosome 1 a LOD=3.7 was found at marker interval D1S1597–D1S407 (29.93–33.75 cM) in Mexican American patients with gall bladder disease; 46% of these patients also had type 2 diabetes. When this group was analysed without diabetic individuals, that locus disappeared, possibly indicating that components related to type 2 diabetes are important genetic or pathogenetic factors [10]. Other studies on parameters of lipid metabolism (LOD=3.1, D1S2826–D1S513, 41.92–60.01 cM) and on hypertension in Australian sib pairs (LOD=3.1, D1S2834–D1S2728, 31.02–33.75 cM) also reported linkage here [10, 11]. In nondiabetic Mexican Americans body size-adiposity showed suggestive evidence of linkage at D1S1597 (LOD=2.53, 29.93 cM) [12]. At D1S1597 Iwasaki et al found nominal evidence for linkage (LOD=0.77, p=0.030) in an autosomal genome scan for type 2 diabetes genes and genes affecting BMI in Japanese people (164 families, 256 affected sib pairs) [13]. Considering our results, the results from those studies and the fact that the duration of diabetes and poor blood sugar control are strongly associated with gall bladder disease, the existence of a potential diabetes/metabolic syndrome locus seems possible.

Our locus on chromosome 16 was among the linkage signals in a genome-scan meta-analysis approach including

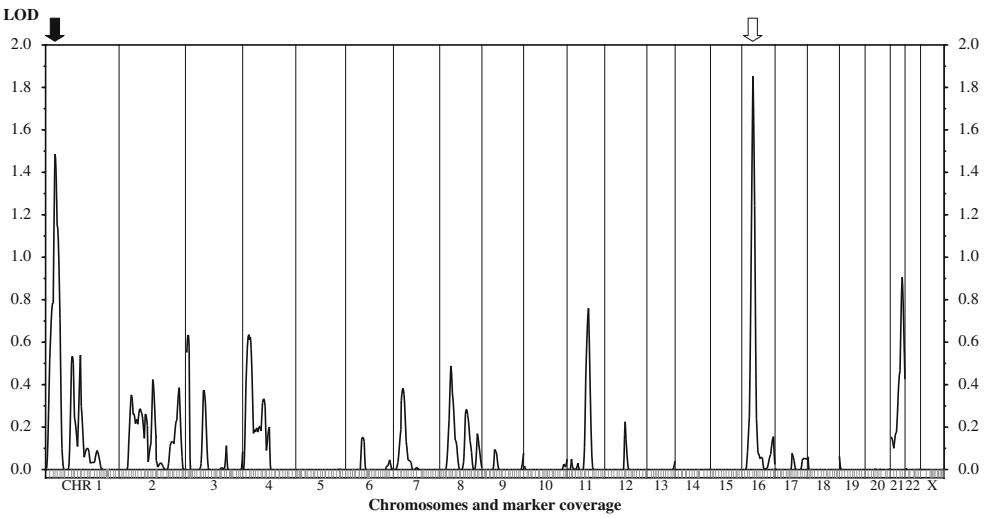

Fig. 1 Nonparametric multipoint LOD plot (MERLIN) using a linear allele-sharing model. *Black arrow*, locus on chromosome 1; *open arrow*, locus on chromosome 16. *Vertical grey lines* below the *x*-axis show the relative positions of typed markers

four European genome scans (Botnia I, Botnia II, British, French samples) [14]. The two Botnia samples (LOD=0.78/1.41) and the pooled data (LOD=1.09), but not the British and French samples mapped type 2 diabetes to our region (among other loci).

Neither of the two loci on chromosomes 1 and 16 reached genome-wide significance; however, D16S403 crossed the empirically obtained threshold of "suggestive" significance of LOD=1.51, while D1S3669 (LOD=1.49) was very close to that level (ESM Table 3). According to our simulations, the levels for suggestive/significant evidence for linkage shifted from LOD=2.20/3.63 [15] to LOD=1.51/2.74, due to the difference between the actual amount of information extracted from our genome screen (which was conducted without parental information and using an infinitely dense screen with perfectly informative markers).

We tested locus interactions by performing conditional linkage analyses as described recently [2]. Here, we found no evidence for interaction between the identified loci on chromosomes 1 and 16. However, the conditioning on D16S403 revealed an interaction with the region around D17S2180 (ESM Table 4). The strongest findings from a meta-analysis of four European genome scans were close to this region; this meta-analysis also highlighted the region on chromosome 16 that we also identified, as noted above [14]. Finally, conditioning on D17S2180 led to a ΔLOD of our findings at D1S3669 and D16S403 which indicated interaction between those loci. Interestingly, another broad region on chromosome 1 (170–200 cM) that was highlighted in these interaction analyses is one in which several other genome scans reported evidence for linkage [16–19].

Our study was the first to investigate German type 2 diabetic patients in a genome-wide linkage scan. However, diabetes as a subphenotype was studied in a German sample with myocardial infarction or coronary revascularisation procedure (diabetes in 16.3% of 618 affected sib pairs with myocardial infarction, diabetes in 8.4% of 238 unaffected sibs) revealing an LOD=2.96 at chromosome 6 (D6S1277, 173.31 cM) [20]. In our sample no positive linkage signal was seen in this region, possibly due to the different sample composition with respect to myocardial infarction and population substructure. Several studies have reported a locus around the MODY1 region on chromosome 20 [21–24]. In our sample we were unable to replicate either those findings or the region with recent association results around this locus. Also, we found no evidence for linkage on chromosome 2q37, suggesting that the heavily studied calpain-10 region does not appear to play a major role in the pathogenesis of type 2 diabetes in Germans. Similarly, we found no evidence for linkage to the region around DG10S478, where compelling associations with type 2 diabetes in Icelandic, Danish and US cohorts have been reported [25].

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