Brief Report

Quantitative Trait Loci for Fasting Glucose in Young Europeans Replicate Previous Findings for Type 2 Diabetes in 2q23–24 and Other Locations

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Long before reaching diagnostic cutoff levels for type 2 diabetes, fasting glucose can be a powerful risk marker for this disease. We conducted a genome-wide search for fasting glucose as a quantitative trait in 412 young European sib-pairs including obese children, with adjustment for sex, age, and BMI. We identified more quantitative trait loci specific to fasting glucose and more significant than would be found by simple chance estimated by permutation tests. The strongest linkage was on chromosome 2q (logarithm of odds [LOD] = 3.00) in a region previously linked to type 2 diabetes as a disease. We also found linkage signals of fasting glucose with 7q (LOD = 2.03), 8q (1.28), 17p (2.12), 17q (1.4), and 11p (1.33). These findings suggest that the quantitative genetics of fasting glucose could contribute to the search for type 2 diabetes genes. *Diabetes* 56:1742 –1745, 2007

Since fat accumulation affects glucose homeostasis, obese patients are at risk for impaired glucose tolerance, impaired fasting glucose, and type 2 diabetes. This risk is, however, widely variable across obese patients. While almost all obese adolescents have normal fasting glycemia (1), many but not all develop type 2 diabetes in later adulthood (1,2). Even a slight elevation of fasting glucose seems a strong long-term predictor of type 2 diabetes in young obese (3) and in nonobese (4) adults. In the general population, heritability for fasting glucose varies between 17 and 51% (5,6). Understanding the genetic predisposition to obesity-related hyperglycemia can be facilitated if genetic and phenotypic heterogeneity are limited by studying relatively homogeneous populations and if reliable phenotypes predicting type 2 diabetes (7) are measured in strictly controlled clinical conditions. To localize quantitative trait loci (QTLs) implicated in fasting glucose variability, we carried out a classic microsatellite-based genome-wide scan in 220 multiplex families (964 individuals and 412 sib-pairs) having a relatively high prevalence of obesity and type 2 diabetes.

Plasma glucose concentration is one of the most exquisitely regulated metabolic traits in humans, and its variations are known to be more dependent on nutritional and physiological conditions than on interindividual variance. Beyond the risk of obesity-related type 2 diabetes, there are thus several reasons for electing to study the quantitative genetics of fasting glucose in young obese patients who have been gaining weight continuously and have stayed in the hospital for 3 days' initial check-up. First, their glucose homeostasis can be studied in stable conditions, including minimization of puncture stress, controlled diet, lack of recent dietary or weight changes, normal physical activity, and precise duration of fasting, a protocol aiming at reducing the ratio of experimental variance to genetic variance of glycemia. Studying the dynamic phase of obesity is important for the quality of the phenotype because later evolution of obesity is exposed to medical or dietary interventions and changing fatness, which can alter the natural history of the trait, as well as to metabolic and hormonal dysfunctions created by the long-term exposure to the obese status itself and aging.

Youth is a period of life favorable to the research of genetic factors implicated in the normal variation of glycemia, since evolutionary selection of many metabolic processes of physiological importance take place during youth; these include energy metabolism during pregnancy and lactation, fetal growth, neonatal survival, adaptation to episodic starvation, and provision of fuel to the large human brain mass. Darwinian views postulate that selection forces for fitness and survival are exerted on young individuals until they reproduce. Therefore, studying individual genotype-phenotype variations during youth matches to our research goals.

In addition, juvenile obesity provides us with the opportunity to maximize the interindividual range of variation of fasting glycemia. We thought that maximizing the variance of the studied phenotype was propitious to genetic association studies. Using the birth places of grandparents, we were also careful at selecting individuals from closely related European geographic areas, which minimizes demographic variations in the genetic background.

The 740 children or adolescents included in the linkage analysis were 13.2 ± 0.2 years old and had a BMI of 20.5 ± 0.4 kg/m². Fifty-one percent were female. Fasting glucose showed a near-normal continuous distribution with a mean value of 4.57 ± 0.62 mmol/l compared with 4.53 ±
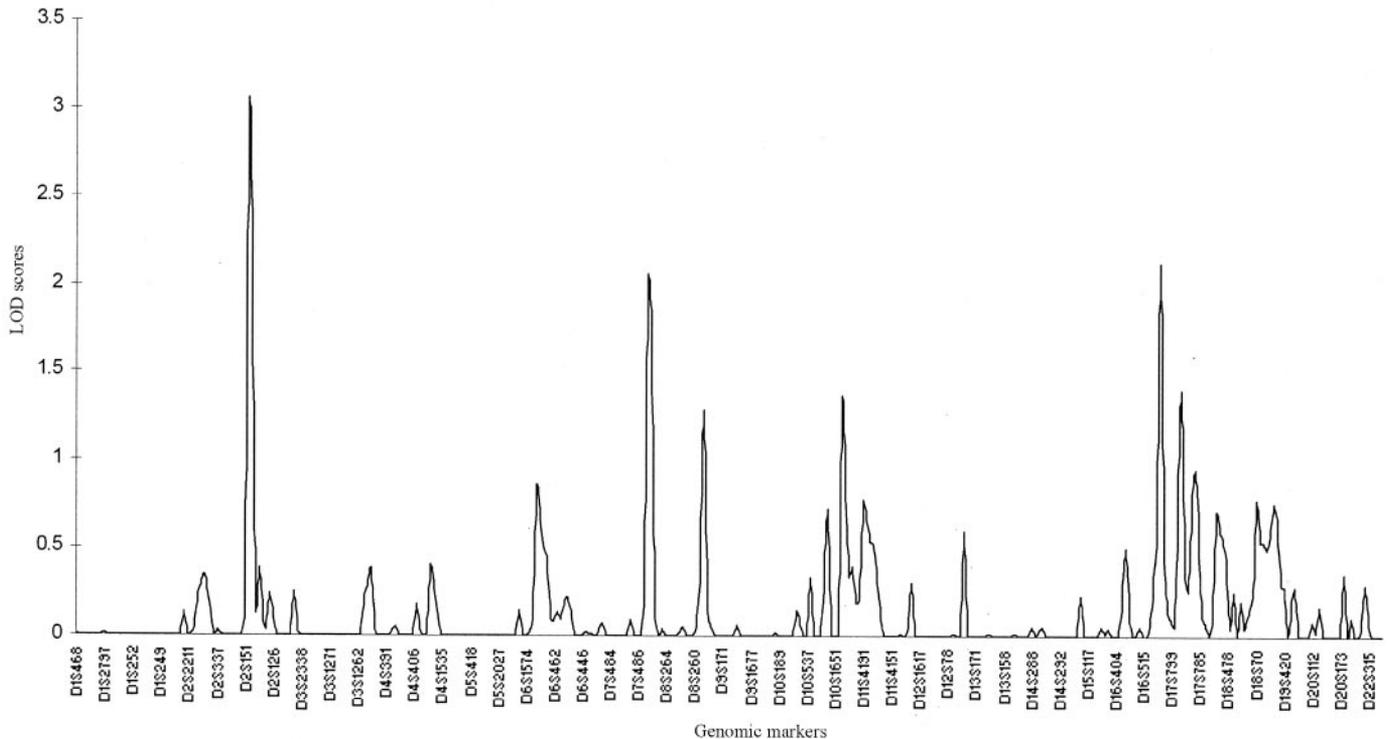
FIG. 1. Graph of the multipoint LOD scores from the genome-wide scan in the 412 Caucasians sibling pairs with 418 DNA markers, using the variance component analysis. The *x*-axis represents few markers in all autosomes, whereas the *y*-axis represents the multipoint LOD score.

0.48 mmol/l in 283 nonobese children from the same population background. There was no significant difference between boys (4.59 ± 0.54 mmol/l) and girls (4.56 ± 0.68 mmol/l). Twelve obese children had impaired fasting glucose with fasting glucose levels between 6.1 and 6.9 mmol/l. Fasting glucose showed a widely dispersed correlation with BMI ($r = 0.23$, $P < 0.001$) and with age ($r = 0.194$, $P < 0.001$), while pubertal maturation did not change fasting glucose in obese adolescents.

We conducted separate and adjusted genome scans for the two quantitative traits, fasting glucose and BMI. Results of linkage analysis are shown in Fig. 1 and Table 1. A total of 10,000 simulations indicated that six peaks with a logarithm of odds (LOD) score ≥1 would be expected by chance only, one with a LOD score ≥2, and none with a LOD score ≥3, while the current genome scan for fasting glucose found five loci with a LOD score between 1 and 2, two loci with a LOD score between 2 and 3, and one locus with a LOD score of 3.

We detected no loci that could be QTLs for current BMI, since no LOD score for this trait was >1, nor did we replicate reports of regions linked to BMI in Caucasian adults (8–11) or children (12–14).

We found several QTLs for fasting glucose that have no detectable effect on BMI. The strongest evidence for linkage to fasting glucose was on 2q with an unadjusted LOD score of 1.78 on 2q23 (D2S142) near NIDDM1 (D2S125–D2S140). This microsatellite revealed links with fasting glucose since the LOD score increased to 3.00 after adjustment to BMI (1.78 to 3.00). This QTL has previously been identified as a type 2 diabetes locus in French (15) and Australians of European origin (16) (Table 1). This consistency suggests the location of one or several glucose QTLs in this vast region. We also identified two other QTLs specific to fasting glucose on 17p13.3 (LOD = 2.12 on

marker D17S831) and 17q12 (LOD = 1.40 on marker D17S798).

Another category of QTLs influence both glycemia and BMI. On chromosome 7q, we found peak evidence for linkage of fasting glucose to D7S684 (7q34, LOD = 2.03) and to D7S661 (7q35–q36, LOD = 1.7), within an ~10-cM interval (Table 1). On chromosome 8, a LOD score of 1.28 was found in D8S514 (8q24). Linkage to type 2 diabetes was previously found in this region (Table 1) (17). On chromosome 11, we found nominal evidence for linkage of fasting glucose at marker D11S4046 (11p15, LOD = 1.33), another region previously linked to type 2 diabetes (18) (Table 1).

In conclusion, this genome-wide analysis supports the existence of QTLs for fasting glucose on the long arm of chromosomes 2, 7, 8, and 17 and the short arm of chromosomes 11 and 17. In the absence of highly significant scores of linkage—not an unexpected result for a complex and highly regulated trait—these observations call for replication of linkage, including additional studies in Caucasian Europeans or in different populations (Table 1). It should be remembered, also, that whole genome linkage or association is underpowered for identifying common genetic variants that have modest effects on diseases or traits (19), in contrast with locus-specific association (20). The glucose QTLs reported here could be of relevance in only obese juveniles, in whom it may help to predict type 2 diabetes, and of minor if any importance in the nonobese population. That our QTLs replicate several type 2 diabetes localizations in cohorts of European ancestry (Table 1) supports the validity of a linkage analysis of glycemia as a quantitative surrogate for type 2 diabetes. That other QTLs for common type 2 diabetes were not found in our young obese study may be because additional genetic factors are necessary to generate sus-

TABLE 1
Regions displaying multipoint LOD scores ≥1 in the current cohort and other studies of fasting glucose or type 2 diabetes

LOD scores for fasting glucose

Chr.	Markers	Regions	Nonadjusted	Adjusted for age and sex	Adjusted for age, sex, and BMI	Candidate genes
2	D2S367	2p21	1.00			PRKCE, SOS1, ARHQ
	D2S2259	2p16	1.15			
	D2S142	2q23	1.78	2.07	3.00	GPD2, IGRP
	D2S2330	2q24.1	1.61	2.00	1.98	
6	D6S257	6p12–p11	1.11			
7	D7S640	7q33	1.66	1.18		
	D7S684	7q34	2.09	2.03	2.03	
	D7S661	7q35	1.58	1.39	1.70	
8	D8S514	8q24.13	1.66	1.66	1.28	
11	D11S4046	11p15.5	2.02	1.99	1.33	SUR, Kir6.2, INS
	D11S1338	11p15.5	1.68	1.44		
	D11S902	11p15–p13	1.41			
	D11S904	11p14–p13	1.25	1.02		
	D11S4191	11p13–q11	1.28	1.30		
	D11S1314	11q13–q14	1.49	1.35		
	D11S937	11q13–q14	1.27			
17	D17S831	17p13.3			2.12	GLUT4
	D17S798	17q12			1.40	

Results from other studies

LOD scores	Phenotypes and populations	Reference
2.28 at 2p21–p16	Type 2 diabetes in Europeans	Vionnet et al. (15)
1.25 at 2q23–q24	Type 2 diabetes in Europeans	Vionnet et al. (15)
2.61 at 2q24	Type 2 diabetes in Australians	Busfield et al. (16)
1.41 at 8q24	Type 2 diabetes in the U.K.	Wiltshire et al. (17)
0.9 at 11p15.1	Type 2 diabetes in the Netherlands	Van Tilburg et al. (18)
11p15.1	Fasting glucose in the Framingham Heart Study	Chen et al. (25)

ARHQ, Ras homolog gene family, member Q; Chr., chromosome; GPD2, mitochondrial glycerophosphate dehyfrogenase; IGRP, islet-specific glucose-6-phosphate catalytic subunit–related protein; INS, insulin; PRKCE, protein kinase C epsilo; SOS1, son of sevenless drosophila homolog 1; SUR, sulfonylurea receptor.

ceptibility to type 2 diabetes later in life. In summary, our study suggests that the complex predisposition of juvenile obesity to type 2 diabetes might benefit from the study of continuous glucose– or insulin-related traits.

RESEARCH DESIGN AND METHODS

Given the known importance of ethnic factors in the regulation of insulin secretion and sensitivity or other physiological or dietary regulators of glucose homeostasis, we selected families of European origin (assessed by the four grandparents' birth places within central or western Europe) to reduce genetic heterogeneity. Families living in Ile-de-France were recruited in Saint Vincent de Paul hospital, where a proband child was followed for obesity, with 29% of family members having obesity and 16% having type 2 diabetes. Clinical research procedures and genetic studies were approved by our institutional review board. Families signed informed consent documents before entering the study and completed a questionnaire about dietary habits, demographic factors, level of education, and family history of weight, height, obesity, and type 2 diabetes. Puberty was assessed using Tanner stages.

Inclusion criteria were *1*) a BMI exceeding the 85th percentile before 6 years of age and reaching the 95th percentile, *2*) the lack of any weight reduction during the course of obesity, *3*) access to sampling of at least one sibling, and *4*) parental data and DNA available.

The study population included 220 families, 154 pedigrees of two sibs, 53 pedigrees of three sibs, 9 pedigrees of four sibs, 3 pedigrees of five sibs, and 1 pedigree of six sibs leading to a total of 412 sib-pairs. The studied sample included 964 individuals. For comparison, we used 283 healthy nonobese age-matched children of European ancestry.

The week preceding the study, obese children were given a weight-maintenance diet containing 250 g carbohydrates/day, then a standardized diet for 3 days. After 12 h of strict overnight fasting, plasma glucose was measured in unstressed conditions using a glucose analyzer (Beckman, Fullerton, CA).

Replication of glucose measurements on following days in 20 children showed an intra-individual variation of <5%. Results are expressed as means ± SD.

Genotyping. A total of 40 ml whole blood was frozen and stored at −20°C. Genomic DNA was extracted with the Gentra Extraction kit (Gentra, Minneapolis, MN).

Genome scan. A genome scan with 418 markers at ∼9-cM intervals was performed in 220 families by the Centre National de Génotypage. The 418 microsatellites were taken predominantly from ankle-brachial pressure index (Applied Biosystem). The linkage mapping set comprises 418 fluorescently labeled PCR primer pairs that define an ∼10-cM resolution human index map. The loci were selected from the Généthon human linkage map based on chromosomal locations and heterozygosity. The map positions were generated from the CEPH genotype data used for the 1996 Généthon map. Full details on these markers and the genotyping procedures can be found at https://products.appliedbiosystems.com/ab/en/US/adirect/ab?cmd = catNavigate2&catID = 600776&tab = Literature.

Before linkage analysis, family structure informations and genomic markers were carefully analyzed to identify incorrect parentship assignment using PedCheck (21). We used the variance components model implemented in Merlin for quantitative traits (22) to evaluate linkage fasting glucose.

The variance component methods ignores detailed aspects of any model underlying the trait mode of inheritance and base inference on the correlation between relatives' similarity with respect to the trait and their similarity with respect to one or more markers. Criteria for significance were based on the lines from Lander and Kruglyak (23) asserting that a LOD score >3.6 indicates genome-wide significance, a LOD score between 2.2 and 3.6 indicates suggestive linkage, and LOD scores between 0.6 and 2.2 are nominal linkage.

We have analyzed genome scan for glycemia adjusted for sex, age, and BMI; for sex and age only; or unadjusted fasting glucose. We have also analyzed BMI adjusted for age and sex.

Simulations. A total of 10,000 simulations were used to determine how many LOD scores over the thresholds of significance would be found by simple

chance using our data and genomic markers. We simulated data using the same marker spacing, allele frequencies, missing data patterns, etc., as the real data, as described by Wiltshire et al. (24), implemented in Merlin. For each simulation sets, we swapped the genotypes versus conserved phenotypes within and among families of similar structure with respect to Mendelian transmission. For the family with six children, we swapped the genotypes within the intrafamilial sibship. Each simulated dataset was analyzed in the same way as for the real dataset. For each simulated analysis, all independent peaks were listed. Thus, we had the number of expected peak by chance for each LOD score.

ACKNOWLEDGMENTS

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