QTLs for fasting glucose in young Europeans replicate previous findings for type 2 diabetes in 2q23-24 and other locations.

Received for publication 29 September 2006 and accepted in revised form 27 February 2007.

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ABSTRACT

Long before reaching diagnostic cut off levels for type 2 diabetes (T2D), 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 Europeans sib-pairs including obese children, with adjustment for sex, age and BMI. We identified more QTLs specific of fasting glucose and more significant than would be found by simple chance estimated by permutation tests. The strongest linkage was on chromosome 2q (LOD=3.00) in a region previously linked to T2D as a disease. We also found linkage signals of fasting glucose with 7q (LOD=2.03), 8q (LOD=1.28), 17p (LOD=2.12), 17q (LOD=1.4) et 11p (LOD=1.33). These findings suggest that the quantitative genetics of fasting glucose could contribute to the search for T2D genes.
INTRODUCTION
Since fat accumulation affects glucose homeostasis, obese patients are at risk for impaired glucose tolerance (IGT), impaired fasting glucose (IFG) and type 2 diabetes (T2D). This risk is however widely variable across obese patients. While almost all obese adolescents have normal fasting glycaemia (1), many but not all develop T2D in later adulthood (1;2). Even a slight elevation of fasting glucose seems a strong long term predictor of T2D in young obese (3) and in nonobese adults (4). In the general population, heritability for fasting glucose varies between 17 and 51% (5;6). Understanding the genetic predisposition to obesity-related hyperglycaemia can be facilitated if genetic and phenotypic heterogeneity are limited by studying relatively homogeneous populations and if reliable phenotypes predicting T2D (7) are measured in strictly controlled clinical conditions. To localize QTLs implicated in fasting glucose variability, we carried out a classical microsatellite-based genome-wide scan in 220 multiplex families (964 individuals and 412 sib-pairs) having a relatively high prevalence of obesity and T2D.

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 inter-individual variance. Beyond the risk of obesity related T2D, 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 stayed in hospital for a three-days initial check-up. First, their glucose homeostasis can then be studied in stable conditions, including minimization of puncture stress, controlled diet, lack of recent dietary or weight changes, normal physical activity, precise duration of fasting, a protocol aiming at reducing the ratio of experimental variance to genetic variance of glycaemia. 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 favourable to the research of genetic factors implicated in the normal variation of glycaemia, since evolutionary selection of many metabolic processes of physiological importance take place during youth such as energy metabolism during pregnancy and lactation, fetal growth, neonatal survival, adaptation to episodic starvation, 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 inter-individual range of variation of fasting glycaemia. 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 year 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 to 4.53 ± 0.48 mmol/L in 283 non obese 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 IFG with fasting glucose 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 Figure 1 and Table 1. Ten thousand simulations indicated that 6 peaks with a LOD score of 1 or greater would be expected by chance only, one with a LOD score of 2 or greater, and none with a LOD score of 3 or greater, 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 equal at 3.

We detected no loci that could be QTLs for current BMI, since no LOD score for this trait greater than 1, neither 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 LOD score increased to 3.00 after adjustment to BMI (1.78 to 3.00). This QTL has previously been identified as a T2D 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 glycaemia 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 a ~10cM interval (Table 1). On chromosome 8, a LOD score of 1.28 was found in D8S514 (8q24). Linkage to T2D 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 T2D (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 are 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 only in obese juveniles where it may help to predict T2D, and of minor if any importance in the non-obese population. The fact that our QTLs replicate several T2D localizations in cohorts of European ancestry (Table 1) supports the validity of a linkage analysis of glycaemia as a quantitative surrogate for T2D. The fact that other QTLs for common T2D were not found in our young obese study is may be due to the fact that additional genetic factors are necessary to generate the susceptibility to T2D later in life. In summary, our study suggests that the complex predisposition of juvenile obesity to T2D might benefit from the study of continuous glucose or insulin related traits.
RESEARCH DESIGN AND METHODS

Subjects.
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 Île-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 T2D. 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 T2D. Puberty was assessed using Tanner stages.

Inclusion criteria were 1) a BMI exceeding the 85th centile before 6 years of age, and reaching the 95th percentile2) the lack of any weight reduction during obesity course, 3) access to sampling of at least one sibling, 4) parental data and DNA available.

The study population included 220 families, 154 pedigrees of 2 sibs, 53 pedigrees of 3 sibs, 9 pedigrees of 4 sibs, 3 pedigrees of 5 sibs and 1 pedigree of 6 sibs leading to a total of 412 sib-pairs. The studied sample included 964 individuals. For comparison, we used 283 healthy non-obese age-matched children of European ancestry.

Procedures.
The week preceding the study obese children were given a weight-maintenance diet containing 250 g of 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, USA). Replication of glucose measurements on following days in 20 children showed an intra-individual variation of less than 5 %. Results are expressed as mean ±sd.

Genotyping.
40ml of whole blood from 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 ~9cM (centimorgans) intervals was performed in 220 families by the Centre National de Génotypage (CNG). The 418 microsatellites were taken predominantly from ABI (Applied Biosystem). The linkage mapping set comprises 418 fluorescently labelled PCR primer pairs that define a ~10cM 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 in 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 Lander’s and Kruglyak’s lines (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 analysed genome scan for glycaemia adjusted for sex, age and BMI, for sex and age only or unadjusted fasting glucose. We have also analysed BMI adjusted for age and gender.
Simulations.
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 6 children, we swapped the genotypes within the intra-familial sibship. Each simulated dataset was analyzed in the same way as for the real dataset. For each simulated analysis all independent peaks was listed. So we had the number of expected peak by chance for each LOD score.

Acknowledgements. DF is supported by the Alfred Jost Research Fellowship of Serono Biopharmaceuticals. The authors thank all technicians and engineers of the Centre National de Génotypage (Evry) whose genotyping work made this study feasible. We thank two anonymous reviewers for suggesting further genome scan analyses.
Reference List


Table 1. Regions displaying multipoint LOD scores of 1 or more in the current cohort and other studies of fasting glucose or T2D.

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Chr. = Chromosome ; PRKCE = Protein Kinase C Epsilo; SOS1 = Son Of Sevenless drosophila homolog 1; ARHQ = Ras Homolog gene family, member Q ; GPD2 = mitochondrial Glycerophosphate Dehydrogenase ; IGRP = Islet-specific Glucose-6-phosphate catalytic subunit Related Protein ; SUR = SulfonylUrea Receptor ; Kir6.2 = Potassium Channel Inwardly Rectifying member 11 ; INS = Insulin and GLUT4 = facilitated Glucose Transporter member 4.
Figure 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.