The 31-cM Region of Chromosome 11 Including the Obesity Gene Tubby and ATP-Sensitive Potassium Channel Genes, *SUR1* and *Kir6.2*, Does Not Contain a Major Susceptibility Locus for NIDDM in 127 Non-Hispanic White Affected Sibships

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Late-onset NIDDM is a polygenic disorder with both genes and environmental factors contributing to susceptibility (1). Various genetic strategies are being used to identify NIDDM susceptibility genes, including linkage studies using large groups of affected sib pairs and association studies with well-matched groups of affected and unaffected subjects. Risch and Merikangas (2) have recently reviewed the merits of these two approaches and they note that affected–sib pair linkage analysis has good power to find genes with major effects but only limited power to detect genes of modest effect.

The ATP-sensitive K^+ channel plays a central role in the regulation of insulin secretion by coupling metabolism to membrane potential. This channel is a heteromeric complex of the type 1 sulfonylurea receptor (SUR1) and the inwardly rectifying K^+ channel (Kir6.2) (3). The genes encoding these two proteins are located adjacent to one another in human chromosome band 11p14.1, and mutations in both SUR1 and Kir6.2 are associated with familial hyperinsulinism, a rare recessive disorder characterized by excessive insulin secretion in the presence of severe hypoglycemia (4). In addition, association studies have identified two variants in SUR1 that are associated with NIDDM in two non-Hispanic white populations from Utah and the U.K. and a two- to threefold increase in relative risk of NIDDM (5). Based on their results, the authors suggested that defects at SUR1 may be a major genetic factor contributing to NIDDM in whites of northern European origin. In contrast, linkage studies in Mexican-American and Japanese affected sib pairs found no evidence for linkage with markers in the region of SUR1, indicating that it was not a major NIDDM susceptibility gene in these populations (6,7). We have tested eight markers spanning a 31-cM region of chromosome 11 band 11p15.1-p14, which includes SUR1, the linked Kir6.2 gene, and the human homolog of the murine obesity locus tubby (8,9), for linkage with NIDDM in a group of 159 non-Hispanic white affected sib pairs.

Patients were defined as having NIDDM according to the criteria of the World Health Organization or the National Diabetes Data Group (10). Only subjects with onset of NIDDM after 35 years of age were included. Informed consent was obtained from all subjects before testing. These studies were approved by the University of Chicago Institutional Review Board and by the University Clinic Carl Gustav Carus Dresden Ethics Committee. The Dresden group was ascertained through the diabetes clinic of the Department of Internal Medicine III (University Clinic Carl Gustav Carus Dresden) and consisted of 200 individuals from 96 families, for a total of 113 affected sib pairs. The Chicago group was ascertained through the diabetes clinic at The University of Chicago clinics and hospitals and consisted of 69 individuals from 31 families, for a total of 46 affected sib pairs. The combined group consisted of 269 individuals from 127 families, for a total of 159 affected sib pairs (114 sibships with 2 affected, 11 sibships with 3 affected, and 2 sibships with 4 affected). Parental affection status of these subjects is unknown. The average age for the Dresden/Chicago groups at the time of study was 59.2 ± 9.4/55.6 ± 12.4 years (mean ± SD) and at diagnosis was 44.9 ± 10.0/47.2 ± 12.4 years. The duration of NIDDM was 14.5 ± 7.9/8.6 ± 7.8 years and BMI was 28.5 ± 4.9/32.4 ± 7.8 kg/m^2. Treatment was 9.0/19.1% with diet, 38.0/36.8% with oral hypoglycemic agents, 51.5/44.4% with insulin, and 1.5/0% unknown. Population differences were tested by Student's t test, assuming unequal variances with 2 df (age at time of study, $P < 0.028$; age at diagnosis, not significant; duration of diabetes, $P < 0.0000007$; and BMI, $P < 0.0005$).

The yeast artificial chromosome (YAC) address for *TUB*, the human homolog of the murine obesity gene tubby, was determined by screening YAC pools (Research Genetics, Huntsville, AL) with the primers TubF, 5′-TTTGCCATTGCCCTGTCCAGCTTC-3′, and TubR, 5′-CAAAGGAGCCAGTTCCTGGCTGGC-3′, as described previously (11). *TUB* was mapped and confirmed to YACs 954F4 and 960F10. These

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Kir6.2, inwardly rectifying K^+ channel; LOD, logarithm of odds; MLS, maximum likelihood score; PIC, polymorphism information content; STRP, simple tandem repeat polymorphism; SUR1, type 1 sulfonylurea receptor; YAC, yeast artificial chromosome.

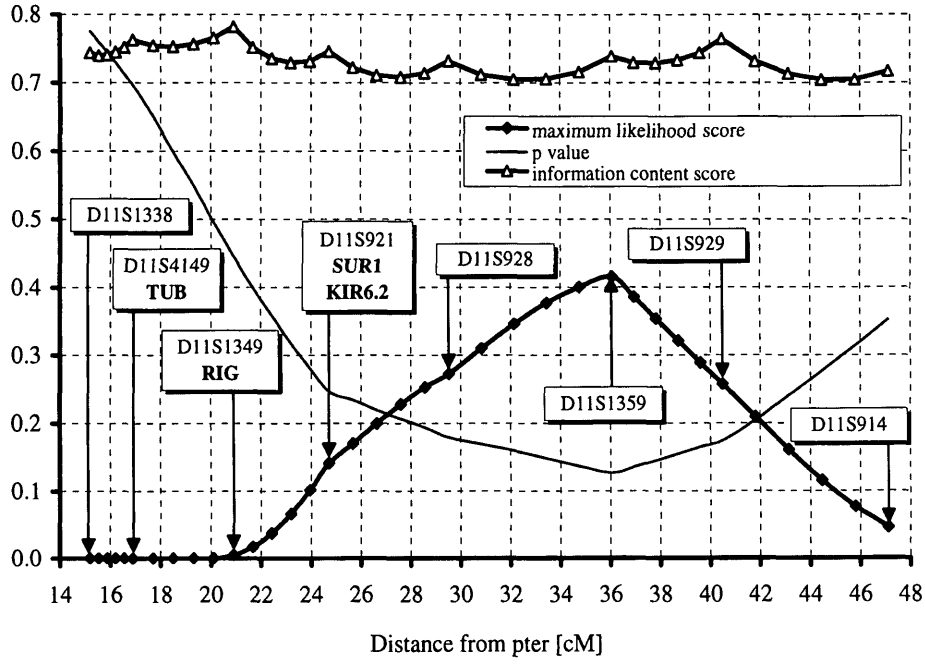

FIG. 1. Multipoint analyses. The results of the semiparametric multipoint linkage analyses of the 31-cM region of chromosome 11, including *TUB*, the ATP-sensitive potassium channel genes, *SUR1*, and *Kir6.2*, are shown. The location of the human homolog of the rat insulinoma gene *RIG* is also indicated (19).

YACs included the simple tandem repeat polymorphism (STRP) D11S932. The Généthon Human Genetic Linkage Map 1996 indicated that D11S4049 mapped to the same position and this marker was used for linkage studies because of its higher polymorphism information content (PIC) value (12).

Genotyping with the simple-sequence repeat polymorphisms D11S1338, D11S4149, D11S1349, D11S921, D11S928, D11S1359, D11S929, and D11S914 (Research Genetics) was carried out, as described previously (6).

Markers were tested for linkage, as described previously, using the computer programs AFFSIB (13), SPLINK (14,15), and GENEHUNTER (16; modified by A. Kong, N.J.C., unpublished observations); all families were weighted equally, regardless of the number of affected sibs. Marker allele frequencies were estimated from the data using SPLINK. To insure that heterogeneity between the American and German samples did not affect the results, the two groups were analyzed separately as well as in a pooled Caucasian sample.

Eight STRPs spanning a 31-cM region of chromosome 11 were tested for linkage with NIDDM in a combined group of 159 non-Hispanic affected sib pairs. There was no evidence of linkage (i.e., $P < 0.05$) of any of these markers with NIDDM using any of the tests described above; the results of the multipoint analysis using the program GENEHUNTER are shown in Fig. 1. The results were similar whether each study group was analyzed independently or combined, suggesting that even when differences in ethnic background were present in the Dresden/Chicago samples, they did not confound the analysis. The marker D11S1359 almost reaches the threshold for nominal evidence for linkage (i.e., $P < 0.05$) with a value of the maximum likelihood score (MLS) (SPLINK) of 0.62 ($P = 0.06$).

The multipoint results provide no compelling evidence for linkage of markers for *TUB* (D11S4149) or *SUR1/Kir6.2* (D11S921) with NIDDM, excluding these as major susceptibility genes in this group of non-Hispanic whites.

The multipoint analyses permit us to quantify the possible effect of loci from this region on NIDDM susceptibility. The highest multipoint logarithm of odds (LOD) score is in the region of D11S1359, not *TUB* or *SUR1/Kir6.2*, and the magnitude of its effect can be quantified based on the locus-specific λ_s value (17), which for D11S1359 is 1.18. Assuming a total λ_s of 3.5 for NIDDM (18) and a multiplicative model, a susceptibility locus from this region accounts for <10% of the familial clustering of NIDDM. The results presented in Fig. 1 indicate that *TUB* and *SUR1/Kir6.2* would have an even smaller effect. Loci with λ_s of ≥ 2.8 can be excluded from this region (LOD scores –2.0 or less).

In summary, the results provide little evidence for linkage of *SUR1/Kir6.2* or *TUB* with NIDDM. The strongest evidence for linkage, albeit nonsignificant, was at D11S1359, ~1 cM from D11S899, which had a maximum LOD score of 3.37, with 2-h glucose concentration in Mexican-Americans (20). If there is a diabetes susceptibility locus in this region, it is likely to account for only a small portion of the total liability to NIDDM in this non-Hispanic white population. The major loci contributing to NIDDM susceptibility in this population remain to be determined.

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REFERENCES

1. Turner RC, Hattersley AT, Shaw JTE, Levy JC: Type II diabetes: clinical aspects of molecular biological studies. *Diabetes* 44:1–10, 1995
2. Risch N, Merikangas K: The future of genetic studies of complex human diseases. *Science* 273:1516–1517, 1996
3. Inagaki N, Gonoi T, Clement JP IV, Namba N, Inazawa J, Gonzalez G, Aguilar-Bryan L, Seino S, Bryan J: Reconstitution of IKATP: an inward rectifier sub-

unit plus the sulfonylurea receptor. *Science* 270:1166–1170, 1995
4. Thomas PM, Ye Y, Lightner E: Mutation of the pancreatic islet inward rectifier Kir6.2 also leads to familial persistent hyperinsulinemic hypoglycemia of infancy. *Hum Mol Genet* 11:1809–1812, 1996
5. Inoue H, Ferrer J, Welling CM, Elbein SC, Hoffman M, Mayorga R, Warren-Perry M, Zhang Y, Millns H, Province M, Bryan J, Permutt MA, Aguilar-Bryan L: Sequence variants in the sulfonylurea receptor (SUR) gene are associated with NIDDM in Caucasians. *Diabetes* 45:825–831, 1996
6. Iwasaki N, Kawamura M, Yamagata K, Cox NJ, Karibe S, Ohgawara H, Inagaki N, Seino S, Bell GI, Omori Y: Identification of microsatellite markers near the human genes encoding β-cell ATP-sensitive K^+ channel and linkage studies with NIDDM in Japanese. *Diabetes* 45:267–269, 1996
7. Stirling B, Cox NJ, Bell GI, Hanis CL, Spielman RS, Concannon P: Linkage studies in NIDDM with markers near the sulfonylurea receptor gene. *Diabetologia* 38:1479–1481, 1995
8. Noben-Trauth K, Naggert JK, North MA, Nishina PM: A candidate gene for the mouse mutation tubby. *Nature* 380:534–538, 1996
9. Kleyn PW, Fan W, Kovats SG, Lee JJ, Pulido JC, Wu Y, Berkemeier LR, Misumi DJ, Holmgren L, Charlat O, Woolf EA, Tayber O, Brody T, Shu P, Hawkins F, Kennedy B, Baldini L, Ebeling C, Alperin GD, Deeds J, Lakey ND, Culpepper J, Chen H, Glücksmann-Kuis MA, Carlson GA, Duyk GM, Moore KJ: Identification and characterization of the mouse obesity gene tubby: a member of a novel gene family. *Cell* 85:281–290, 1996
10. Harris MI: Classification, diagnosis criteria, and screening for diabetes. In *Diabetes in America*, 2nd ed. Washington, DC, U.S. Govt. Printing Office, 1995, p. 15–36 (NIH publ. no. 95-1468)
11. Gambino V, Menzel S, Trabb JB, Xiang KS, Lindner T, Louït A, Chen E, Mereu LE, Furuta H, Iwasaki N, Kawamura M, Omori Y, Rietzsch H, Schulze J, Schröder HE, Concannon P, Hanis CL, Spielman RS, Yamagata K, Cox NJ, Bell GI: An approach for identifying simple sequence repeat DNA polymorphisms near cloned cDNAs and genes: linkage studies of the islet amyloid polypeptide/amylin and liver glycogen synthase genes and NIDDM. *Diabetes* 45:291–294, 1996
12. Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Marc S, Hazan J, Seboun E, Lathrop M, Gyapay G, Morissette J, Weissenbach J: A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 380 (Suppl.):152–154, 1996
13. Hanis CL, Boerwinkle E, Chakraborty R, Ellsworth DL, Concannon P, Stirling B, Morrison VA, Wapelhorst B, Spielman, RS, Gogolin-Ewens KJ, Shephard JM, Williams SR, Risch N, Hinds D, Iwasaki N, Ogata M, Omori Y, Petzold C, Rietzsch H, Schröder HE, Schulze J, Cox NJ, Menzel S, Boriraj VV, Chen X, Lim LR, Lindner T, Mereu L, Wang YQ, Xiang K, Yamagata K, Yang Y, Bell GI: A genome-wide search for human non-insulin-dependent (type 2) diabetes genes reveals a major susceptibility locus on chromosome 2. *Nature Genet* 13:161–167, 1996
14. Holmans P: Asymptotic properties of affected-sib-pair linkage analysis. *Am J Hum Genet* 52:362–374, 1993
15. Holmans P, Clayton D: Efficiency of typing unaffected relatives in an affected-sib-pair linkage study with single-locus and multiple tightly linked markers. *Am J Hum Genet* 57:1221–1232, 1995
16. Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES: Parametric and nonparametric linkage analysis: a unified multipoint approach. *Am J Hum Genet* 58:1347–1363, 1996
17. Risch N: Assessing the role of HLA-linked and unlinked determinants of disease. *Am J Hum Genet* 40:1–14, 1987
18. Rich SS: Mapping genes in diabetes: genetic epidemiological perspective. *Diabetes* 39:1315–1319, 1990
19. Shiga K, Yamamoto H, Okamoto H: Isolation and characterization of human rig and its pseudogenes: the functional gene has features characteristic of housekeeping genes. *Proc Natl Acad Sci USA* 87:3594–3598, 1990
20. Stern MP, Duggirala R, Mitchell BD, Reinhart LJ, Shivakumar S, Shipman PA, Uresandi OC, Benavides E, Blangero J, O'Connell P: Evidence for linkage of regions on chromosome 6 and 11 to plasma glucose concentrations in Mexican Americans. *Genome Res* 6:724–734, 1996