Prohormone Convertase 1 in Obesity, Gestational Diabetes Mellitus, and NIDDM

No Evidence for a Major Susceptibility Role

Kamini Kalidas, Eleanor Dow, Philip J. Saker, Nicholas Wareham, David Halsall, Robert S. Jackson, Siew-Pheng Chan, Susan Gelding, Mark Walker, Eleni Kousta, Desmond G. Johnston, Stephen O'Rahilly, and Mark I. McCarthy

Improved understanding of the primary molecular events underlying NIDDM and obesity is essential if more effective therapies are to be devised. Individual susceptibility to these interrelated conditions is under genetic influence (1), and clues to the identity of the major aetiological genes may come from physiological studies that pinpoint candidate pathways. A characteristic feature of NIDDM and certain prediabetic states, such as gestational diabetes mellitus (GDM), is a marked increase in the proportion of circulating insulin precursor molecules (proinsulin and split proinsulin intermediates) (2,3). Release of disproportionate amounts of these biologically inactive precursors may contribute to the relative insulin deficiency apparent in GDM and NIDDM (2).

The processing of proinsulin to mature insulin is catalyzed by prohormone convertase (PC) enzymes active in β-cell granules. PC1 (also named PC3) cleaves intact proinsulin to produce 32,33 split proinsulin. PC2 and carboxypeptidase E (CPE) catalyze subsequent reactions (4). Functional defects in any of these candidate genes could contribute to the NIDDM phenotype.

Furthermore, recent studies indicate a role for these loci in the determination of obesity. Mutations in the Cpe gene resulting in absent enzyme activity in islets and pituitary underlie the phenotype of the fat/fat mouse (5). More recently, Jackson et al. (6) reported on a family segregating two distinct PC1 mutations. The mother was a compound heterozygote who had presented with childhood obesity, GDM, and a variety of endocrine abnormalities attributable to defective prohormone processing. In this report, we have sought to establish whether variation in the PC1 gene contributes to typical NIDDM, GDM, and obesity.

First, we sought evidence for linkage between the PC1 gene region and NIDDM in 26 families (13 European, 10 South Asian Indian, 3 Black-Caribbean) ascertained in London, Newcastle-upon-Tyne, and Malaysia. In all families, at least three members were diabetic (7). Median (range) BMI for the diabetic individuals was 27.2 (18.2–50.0) kg/m².

The Genebridge 4 radiation hybrid panel was used to localize the PC1 gene to a 4-cM interval between AFM205wg7 and GATA48A11 on chromosome 5q (logarithm of odds [LOD] score >3) by polymerase chain reaction (PCR) amplification of sequences in exons 8 and 11 (8). We selected five microsatellite markers spanning a 15-cM region of chromosome 5q15-21 centered on PC1 (Table 1): D5S401-7 cM-AFM205wg7-4 cM-GATA48A11-2 cM-D5S409-2 cM-D5S433. Primer sequences and interlocus distances were taken from Genethon (9) and Whitehead (version 11.9) databases (10). Genotypes were determined by acrylamide gel electrophoresis after radioactive PCR (using [35S]dATP).

In the absence of a validated segregation model for NIDDM, we report the multipoint, nonparametric results obtained with GENEHUNTER (Table 1). Marker allele frequencies were estimated from founders and were similar for all ethnic groups. We found no evidence for excess allele-sharing in the region: the maximum nonparametric linkage (NPL) score obtained was 0.24 at D5S409. Reanalysis, after subdivision by pedigree origin, did not indicate ethnic heterogeneity (additional information about this subject can be found in the on-line appendix at www.diabetes.org/diabetes/appendix.htm).

As linkage analysis is insensitive to minor genetic effects, we screened the PC1-coding region to determine the prevalence of novel and previously reported variant sequences. We examined four subject groups:

1. From the families described, 80 diabetic individuals, 25 unaffected spouses, and 10 nondiabetic family members with the highest proinsulin levels (11) were examined.
2. Nondiabetic subjects with high intact:split proinsulin levels recruited from the population-based Isle of Ely Diabetes Study (12) were studied, and of 1,071 glucose-tolerant individuals surveyed, we selected 17 with the highest fasting intact:split proinsulin ratio (13). All were Caucasian, four

From the Imperial College School of Medicine at St. Mary's (K.K., P.J.S., E.K., D.G.J., M.I.M.), London; Ninewells Hospital (E.D.), Dundee; Addenbrookes Hospital (N.W., D.H., R.S.J., S.O.R.), Cambridge; London Hospital Medical School (S.G.), London; University of Newcastle (M.W.), Newcastle-upon-Tyne, U.K.; University Hospital (S.P.C.), Kuala Lumpur, Malaysia.

Address correspondence and reprint requests to Mark McCarthy, Unit of Metabolic Medicine, St. Mary's Hospital, London, W2 1NY, U.K. E-mail: m.mccarthy@ic.ac.uk.

Received for publication 30 July 1997 and accepted in revised form 22 October 1997.

Additional information can be found in an on-line appendix at www.diabetes.org/diabetes/appendix.htm.

CPE, carboxypeptidase E; GDM, gestational diabetes mellitus; LOD, logarithm of odds; NPL, nonparametric linkage; PC, prohormone convertase; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SSCP, single-strand conformation polymorphism.
were male, BMI was 25.4 (19.5–30.6) kg/m² and WHR 0.79 (0.70–1.01) [median (range)].

3. Obese, diabetic individuals taken from the British Diabetic Association–Warren 2 NIDDM repository, in which all families include, at minimum, an affected sibpair of exclusively British/Irish origin were also studied. We selected ten female diabetic individuals with the highest BMIs from the St. Mary's cohort. BMI was 39.9 (38.5–48.9) kg/m² and WHR was 0.89 (0.78–0.91).

4. A total of 12 obese women with GDM (2-h glucose >7.8 nmol/l) recruited from antenatal clinics at the Royal London Hospital or St Mary's, London were included in this study. Four were European and eight were Bengali. BMI was 33.8 (32.3–45.7) kg/m². WHR was not recorded. Informed consent was obtained from all subjects.

All 14 exons were amplified using published primer sequences and conditions (8) and variants were sought using heteroduplex and single-strand conformation polymorphism (SSCP) analyses. For heteroduplex analysis, PCR products were denatured, cooled to room temperature, and electrophoresed on 0.5 × MDE gels (Flowgen, Lichfield, UK). For SSCP analysis, denatured PCR products were electrophoresed on 10% nondenaturing polyacrylamide gels at 4°C and 25°C. Known variants in the glucokinase and uncoupling protein (UCP1) genes were reliably detected by both methods, as was the previously described exon 14 polymorphism (8). No novel sequence variants were detected in any population group.

The study groups were also tested explicitly for four known PC1 variants. Two of these variants (Arg/Gln53 in exon 2 and Gin/Glu688 in exon 14) had been described in Japanese populations (8); the others (the intron 5 splice donor site A→C84 and Gly/Arg483 in exon 13) were first described by Jackson et al. (6). PCR-restriction fragment length polymorphism (RFLP) analyses were used to genotype for Arg/Gln53 (loss of a Taq I site), Gly/Arg483 (loss of Nla IV site) and Gin/Glu688 (loss of ScrFI site). The intron 5 variant altered no restriction sites, so we developed a robust SSCP assay (primers 5'-TGCTCTTTTAGATCCAGGCG-3' and 5'-CTTATTTTTACAAATGATATTGA-3'). The Arg/Gln53, Gly/Arg483, and intron 5 variants were not seen in any subject studied. The exon 14 variant was found in ~20% of chromosomes in each study cohort (37 of 160 from affected family members, 11 of 50 from unaffected spouses, 4 of 20 from hyperproinsulinemic relatives, 8 of 34 from Ely subjects, 5 of 24 from GDM subjects, and 5 of 20 from obese, diabetic subjects from the Warren 2 collection) and each ethnic group. These prevalences are similar to those reported in Japanese diabetic and control groups (24 and 22%, respectively) (8).

Our findings fail to support a role for variation in the PC1 gene in determining susceptibility to obesity, GDM, and NIDDM in the populations studied. No evidence for excess allele-sharing was observed in our dataset. We note that there have been no reports of linkage in this region in genomewide scans for NIDDM (14,15). Absence of evidence for linkage does not exclude the possibility that variants in the gene contribute to a minority of cases of diabetes and/or obesity. The populations chosen for mutation detection were enriched with individuals considered most likely to have PC1 mutations through a combination of clinical features, family history, and physiological measurements. Nonetheless, no novel mutations were found. Furthermore, previously described polymorphisms were not overrepresented among affected individuals, confirming and extending the findings of other groups (6,8).

We conclude that the alterations in insulin processing observed in prediabetic and diabetic populations are not due to variation in the coding regions of the PC1 gene. The possibility that etiological variants lie outside the screened regions (e.g., cryptic splice sites or regulatory mutations) will require exploration in subsequent studies.

ACKNOWLEDGMENTS
Funding for this work was provided by the British Diabetic Association, the St Mary's Hospital Joint Research Standing Committee, the Anglia and Oxford NHS Research and Development Directorate, and the Medical Research Council.

We thank Barbara Millauer and Henri Mulnier and the physicians, general practitioners, and family members who contributed to the Warren 2 repository as well as Prof. Graham Hitman, Dr. Barbara Boucher, Dr. John P. Monson, and Dr. Trevor Beedham (London) for assistance in collection of patients.

REFERENCES
1. McCarthy MI, Progol P, Hitman GA: The genetics of non-insulin-dependent diabetes mellitus: tools and aims. Diabetologia 37:959–968, 1994
2. Porte D Jr, Kahn SE: Hyperproinsulinaemia and amyloid in NIDDM: clues to etiology of islet-cell dysfunction. Diabetes 38:1333–1336, 1989
3. Dornhorst A, Davies M, Anyaoku V, Hampton SM, Elkeles RS, Beard RW, Johnston DG: Abnormalities in fasting proinsulin concentration in mild gestational diabetes. Clinical Endocrinology 34:211–213, 1991
4. Rhodes CJ, Lincoln B, Shoelson SE: Preferential cleavage of des-31,32-proinsulin over intact proinsulin by the insulin secretory granule type-II processing endopeptidase: implications of a favoured route for prohormone processing. J Biol Chem 267:22719–22727, 1992
5. Naggert JK, Fricker LD, Varlamov O, Nashima PM, Roulle Y, Steiner DP, Carroll RJ, Falgen RJ, Leiter EH: Hyperproinsulinaemia in obese fa/fa mice associated with a carboxypeptidase E mutation which reduces enzyme activity. Nature Genet 10:135–142, 1995
6. Jackson R, Creemers JWM, Ohagi S, Bertagna X, Sanders L, Montague C, Hutton J, O'Rahilly S: A human syndrome of severe obesity and defective prohormone processing associated with mutations in the prohormone convertase 1 gene. Nature Genet 16:303–306, 1997
7. Dow E, Gelding SV, Skinner E, Hewitt JE, Gray IP, Mather H, Williamson R, Johnston DG: Genetic analysis of the glucokinase and the chromosome 20 susceptibility locus in families with type 2 diabetes. Diabetic Med 11:856–861, 1994

| Marker | Genetic distance (cM) | Physical distance (cR) | NPL score | P value |
|--------|----------------------|-----------------------|-----------|---------|
| DSS401 | 7                    | 298.4                 | -0.518    | 0.70    |
| AFM206w7 | 7.7             | 316.9                 | -0.192    | 0.57    |
| AFM206w7 | 7.8              | 316.9                 | -0.134    | 0.55    |
| AFM206w7 | 8.6              | -0.077                | 0.52      |         |
| AFM206w7 | 9.4              | -0.021                | 0.50      |         |
| AFM206w7 | 10.2             | 0.034                 | 0.48      |         |
| GATA48A11 | 11.0          | 328.7                 | 0.088     | 0.45    |
| DSS5409 | 13.0             | 350.3                 | 0.239     | 0.39    |
| DSS5433 | 15.0             | 0.213                 | 0.40      |         |

Radiation hybrid mapping placed amplicons in exons 8 and 11 of the PCI gene at 318.32 cR and 327.45 cR, respectively, in the interval between AFM205w7 and GATA48A11. Marker DSS5433 has not been placed on the radiation hybrid map.cM, centimorgan; cR, centiRay.
8. Ohagi S, Sakaguchi H, Sanke T, Tatsuta H, Hanabusa T, Nanjo K: Human pro-hormone convertase 3 gene: exon-intron organisation and molecular scanning for mutations in Japanese subjects with NIDDM. Diabetes 45:897–901, 1996
9. Dib C, Faure S, Flizes CS, 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 380:153–154, 1996
10. Hudson TJ, Stein LD, Gerety SS, Ma J, Castle AB, Silva J, Slonim DK, Baptista R, Kruglyak L, Xu SH, Hu X, Colbert AME, Rosenberg C, Reeve-Daly MP, Rozen S, Hui L, Wu X, Vestergaard C, Wilson KM, Bae JS, Maitra S, Ganiatsas S, Evans CA, DaAngelis MM, Ingalls KA, Nahf RW, Horton LT, Anderson MO, Collymore AJ, Ye W, Koyoumjian V, Zemsteva IS, Tam J, Devine R, Courtney DF, Renaud MT, Nguyen H, O’Connor T, Flizes CS, Fauré S, Gyapay G, Dib C, Morissette J, Orlien JB, Birren BW, Goodman N, Weissenbach J, Hawkins TL, Foote S, Page DC, Lander ES: An STS-based map of the human genome. Science 270:1945–1954, 1995
11. Gelding SV, Nithyananthan R, Chan SP, Skinner E, Robinson S, Gray IP, Mathur H, Johnston DG: Insulin sensitivity in non-diabetic relatives of patients with non-insulin dependent diabetes from two ethnic groups. Clin Endocrinol 40:55–62, 1994
12. Williams DRR, Wareham NJ, Brown DC, Byrne CD, Clark PMS, Cox BD, Cox LD, Day NE, Hales CN, Palmer CR, Shackleton JR, Wang TWM: Undiagnosed glucose intolerance in the community: the Isle of Ely diabetes project. Diabetic Med 12:30–35, 1994
13. Hales CN, Byrne CD, Petry CJ, Wareham NJ: Measurement of insulin and proinsulin. Diabetes Rev 4:320–335, 1996
14. Haris CL, Boerwinkle E, Chakraborty R, Ellsworth DL, Concannon P, Stirling B, Morrison VA, Wagelhorst B, Spielman RS, Gogolin-Ewens KJ, Shephard JM, Williams SR, Risch N, Hinds D, Iwasaki N, Ogata M, Onori Y, Pezzold C, Ristach H, Schroder HE, Schulze J, Cox NJ, Menzel S, Borrajr VV, Chen X, Lim LR, Lindner T, Mereu LE, 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 13:161–171, 1996
15. Mahtani MM, Widen E, Lehto M, Thomas J, McCarthy M, Bray J, Bryant B, Chan G, Daly M, Forsblom C, Kanninen T, Kirby A, Kruglyak L, Munnelly K, Parkkonen M, Reeve-Daly MP, Weaver A, Brettin T, Duyk G, Lander ES, Groop LC: Mapping of a gene for type 2 diabetes associated with an insulin secretion defect by a genome scan in Finnish families. Nature Genet 14:90–94, 1996