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/m^2.

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 Généthon (9) and Whitehead (version 11.9) databases (10). Genotypes were determined by acrylamide gel electrophoresis after radioactive PCR (using [^{35}S]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:32,33 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/m^2 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/m^2 and WHR was 0.89 (0.78–0.91).

4. A total of 12 obese women with GDM (2-h glucose >7.8 mmol/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/m^2. 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/Gln^{53} in exon 2 and Gln/Glu^{638} in exon 14) had been described in Japanese populations (8); the others (the intron 5 splice donor site A→C^{+4} and Gly/Arg^{483} in exon 13) were first described by Jackson et al. (6). PCR-restriction fragment length polymorphism (RFLP) analyses were used to genotype for Arg/Gln^{53} (loss of a *Taq* I site), Gly/Arg^{483} (loss of *Nla* IV site) and Gln/Glu^{638} (loss of *ScrFI* site). The intron 5 variant altered no restriction sites, so we developed a robust SSCP assay (primers 5′-TGTCTCTTTTAGGATCCAGAGGC-3′ and 5′-CTTTATTTCACACAAATGCATATTGA-3′). The Arg/Gln^{53}, Gly/Arg^{483}, 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).

TABLE 1
Cumulative NPL scores and *P* values for multipoint GENEHUNTER analysis of microsatellite markers across chromosome 5q15-21 in NIDDM pedigrees

Marker	Genetic distance (cM)	Physical distance (cR)	NPL score	*P* value
D5S401	0	298.4	–0.518	0.70
AFM205wg7	7	316.9	–0.192	0.57
	7.8		–0.134	0.55
	8.6		–0.077	0.52
	9.4		–0.021	0.50
	10.2		0.034	0.48
GATA48A11	11.0	328.7	0.088	0.45
D5S409	13.0	350.3	0.239	0.39
D5S433	15.0		0.213	0.40

Radiation hybrid mapping placed amplicons in exons 8 and 11 of the *PC1* gene at 318.52 cR and 327.45 cR, respectively, in the interval between AFM205wg7 and GATA48A11. Marker D5S433 has not been placed on the radiation hybrid map.cM, centimorgan; cR, centiRay.

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.

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