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Identification of genetic markers to 20 NIDDM candidate genes by radiation hybrid analysis

Dear Sir,

We have determined the fine chromosomal localization of 20 candidate genes for non-insulin-dependent diabetes (NIDDM) using radiation hybrid analysis [1] in an effort to identify short tandem repeat markers suitable for the genetic analysis of these genes in disease populations. Loci for which markers were identified include: leptin and its receptor, AMP kinase activated protein kinase, hexokinase II, pyruvate carboxylase, glutamine : fructose 6-phosphate amidotransferase (GFAT), islet regenerating protein, glucose 6-phosphatase, adrenoreceptors α2a, α2b, and α2c, beta3 adrenoceptor, liver mitochondrial carnitine palmitoyltransferase I, ras associated with diabetes (RAD), pancreatic polypeptide Y, neuropeptide Y receptor 5, phenylethanolamine-N-methyltransferase, ATP citrate lyase, pyruvate dehydrogenase kinase 4, and the agouti switch protein. Candidates were chosen because biochemical or physiological evidence suggests they play a role in aspects of metabolism which may be involved in the aetiology of NIDDM.

Primers to candidate genes were developed or otherwise obtained based upon published nucleotide sequences. Primers to GFAT were derived from partial genomic sequence obtained from a P1 clone DMPC-HFF#1-802E1 (Genome Systems Inc., St. Louis, Mo. USA) identified by hybridization to GFAT cDNA consisting of bp 123–2168 of genbank accession number M90516. Primers to leptin were designed to amplify a CTTT tetranucleotide repeat contained within bp 4211–4477 of the gene (genbank accession number D63710). Primer sequences were LEPT1 5′-FAM-TCAACAAACCATTCT-GAGTTC-3′ and LEPT2 5′-TGGGACACATGTTCTCAG-GA-3′. The repeat was found to be highly informative in the 43 unrelated CEPH individuals genotyped (heterozygosity = 0.83) and should thus serve as a good marker to genetically evaluate the contribution of leptin to human disease.

Primer pairs to these genes were then optimized for mapping onto the Stanford G3 and Genebridge GB4 radiation hybrid panels (Research Genetics, Huntsville, Ala., USA). Marker retention data was analysed using RHMAP Version 2.01 for the G3 data or the Whitehead Institute-MIT Center for Genome Research radiation hybrid mapping web sever for the GB4 data. Distance relationships to nearby polymorphic markers were established using the statistical package RHMAPPER and RH2PT [2, 3].

Candidate gene markers in Table 1 are those with reported heterozygosities greater than 60 % and estimated to be not more than two megabases (Mb) (66.7 centirays [Cr]) from the candidate gene based on two-point analysis of Stanford G3 panel retention data. In about half of the cases, markers were determined to be within 1 Mb (33.3 cR) of the gene. For some loci, approximate distances from markers had to be inferred by comparing both G3 and GB4 data because of gaps in the map data available from the European Bioinformatics Institute (EBI) [4]. In addition, a marker was nominated for a candidate gene only after one or more of the following conditions was met: 1) proximity to the marker was confirmed by an independent sequence tag site (STS) within the gene; 2) proximity to the marker was confirmed by an independent radiation hybridization (RH) panel; or 3) the candidate gene and nominated marker were part of a linkage group supported with a two-locus logarithm of odds (LOD) score of at least three using RH2PT.

Incorporation of NIDDM candidate gene markers, such as those reported here, within a genome scan will ensure that genes which play a role in insulin action, obesity, energy and glucose metabolism are evaluated for their role in genetic susceptibility to the disease. RH analysis offers a rapid and precise way to localize genes with respect to anonymous markers or other genes and thus offers an advantage over methods such as meiotic mapping or fluorescent *in situ* hybridization (FISH). Even when polymorphic markers are known to exist within the candidate gene, such as with RAD and hexokinase II, it is often desirable to identify other nearby markers that can be more conveniently included within a genome scan set or that are more informative in a particular study population. RH analysis offers a rapid method of identifying these markers.

Analysis of 20 NIDDM candidate genes by RH analysis has led to the identification of a polymorphic marker within 2cM (~ 2Mb) of each gene. A highly informative marker to leptin has also been identified. These markers will prove to be important tools in the ongoing search for diabetes susceptibility loci.

Yours sincerely,
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Table 1. Candidate gene markers
In order to place candidate genes on the radiation hybrid map, PCR was carried out in 96-well microplates (Polyfiltronics, Rockland, Mass., USA). Each reaction contained 25 ng of G3 or GB4 DNA, 1.0 µmol/l each primer (Research Genetics or Keystone, Menlo Park, Calif., USA), 200 µmol/l dNTPs, 1.0 U Taq Polymerase, and 1 × PCR buffer (Perkin-Elmer, Norwalk, Conn., USA) in a 20 µl total volume. PCR amplification, unless otherwise indicated, consisted of 4 min initial denaturation at 94 °C followed by 35 cycles of 1 min denaturation at 94 °C, 1 min at annealing temperature, and 1 min extension at 72 °C. Markers to candidate loci are those estimated to be within two megabases of the gene as determined by 2-point analysis and having reported heterozygosities of at least 60 %. The primer sequences used for mapping each gene along with the annealing temperature (Ta), amplicon sizes and any modifications to the basic PCR conditions are shown

Marker	Locus	Primers to gene	Ta	PCR conditions	Amplicon size (bp)
D1S246	OBR: Leptin receptor	OBRA: TAATATCAACTTAGGAACTTCTA OBRB: TTCAAATTTGGACTCTGGTT	58	5 % DMSO	226
D1S476	AMPK: AMP kinase	AMPKS: TTTTGATTCCACAACTGCAGAGAG AMPKAS: TAGTGCAATAACAGAAAGAAACTA	60		190
D2S358	GFAT: Glutamine: Fructose 6-phosphate amidotransferase	GFATF: GAATTTATCGTTATTCACAAT GFATR: TTGTTGCACATCCCAACA	58		145
D2S139	REG: Islet regenerating protein	REGC: AGACCAGAACTTGAACCTCCTTCT REGN: AGCATCATCACGGACATTACTCTGC	60		323
D2S286	HK2: Hexokinase II	B1264: ATTTCAAGACTAGCCTAGGC B1265: AACCTAACATAGTACCTGGC	53	40 cycles	110: 162
D2S113	ADR2B: Alpha2b adrenoceptor	ADR2BF: CTGCGCTGCCCCTGTGGGTT ADR2BR: CACAGCTAAGCCGGCAAGGG	60	5 % DMSO	199
D4S412	ADR2C: Alpha2c adrenoceptor	ADR2CF: GGCCCGCTCTTCAAGTTCTTC ADR2CR: GACACTGCCTGAAGCCCCTTCT	65	5 % DMSO	144
D4S393	NPYR5: Neuropeptide Y receptor 5	NPY5F: AATTGAGTAAAACGTTCTG NPY5R: ACACTAAATCAGCTTTAAT	50		267
D7S504	OB: Leptin	LEPTF: AATTGAGATTCTTGGATAGCAAG LEPTR: ACAGAGCCCCCATTTAAATT	55		209
D7S527	PDHK4: Pyruvate dehydrogenase kinase 4	PDHK1: TGAACCCTTGTTTCCTCCAAAACA PDHK2: CTTCAGGATTTGATATAAACTCAGG	60		188
D8S255	ADRB3: Beta3 adrenoceptor	BETA3A: GAACTGGCTAGGTTATGCCAA BETA3B: CCACGGACACATCGCATGCTT	60	10 % DMSO	275
D10S597	ADR2A: Alpha2a adrenoceptor	ADR2AF: GAACCCGGTCATCTACACCA ADR2AR: AAACGCAGAGCAGCAGCGCC	58	5 % DMSO	201
D11S913	PC: Pyruvate carboxylase	PCF: CCTGGAGATCGAGTGATCTTG PCR: GAGGAAAGGACGATGGCTGA	63	5 % DMSO	200
D11S987	CPT1: Cartinitine palmitoyltransferase I	CPT1S: TGAGCGACTGGTGGGAGGAG CPT1A: GCATGAGCCAATCCCCAGAG	60		200
D16S496	RAD: Ras Associated with diabetes	RAD7: ACTCAAAGCCGCAAATACCGT RAD8: GCCAATAGAATTCCACAGTAT	58		356: 401
D17S579	PPY: Pancreatic polypeptide Y	PPBF: CAACATGCTGACCAGGCCTAG PPBR: TAGGACACCATCTTGCCCAG	60		200
D17S931	G6P: Glucose 6-phosphatase	G6P4A: GGAGAGAAACGGAATGG G6P4S: GCCAGGCTCCAACATTT	50		259
D17S800	PNMT: Phenylethanolamine N-methyltransferase	PNMTAF: TTGGCGCAGACCTTCGCCA PNMTAR: TCTCCTAAGGGATGTTGCTC	63	5 % DMSO	223
D17S579	ACLY: ATP citrate lyase	ACLY1: GACCAACATCCACAGGCTAACACC ACLY2: CCTACAATGAGGAAGACCCCATCC	65		174
D20S106	ASP: agouti switch protein	AGOUTIA: CCTCTTACCATTACCCCTGA AGOUTIS: CTAGGTGACTTACCCACAAT	60		238

DMSO, Dimethylsulphoxide

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