A Novel Gene for Neonatal Diabetes Maps to Chromosome 10p12.1-p13
Gabrielle S. Sellick, Christine Garrett, and Richard S. Houlston

We report a genomewide linkage analysis of a large consanguineous family segregating autosomal recessively inherited neonatal diabetes and the identification of a novel neonatal diabetes locus. Neonatal diabetes was characterized by low levels of circulating C-peptide with very low to undetectable levels of insulin in the presence of severe hyperglycemia unresponsive to insulin infusion. A dense genomewide linkage search of the family was undertaken using a first generation 10K single nucleotide polymorphism chip containing 10,044 markers. A region of homozygosity harboring the neonatal diabetes disease gene on chromosome 10p12.1-p13 was identified (multipoint logarithm of odds score 3.25). There is a strong history of type 2 diabetes in carriers of the disease gene. It is likely that chromosome 10p12.1-p13 may harbor a maturity-onset diabetes of the young or type 2 diabetes gene. Diabetes 52: 2636–2638, 2003

Neonatal diabetes is defined as hyperglycemia presenting within the first 6 weeks of life in term infants (1). Neonatal diabetes affects from 1 in 500,000 to 1 in 400,000 live births (2,3). We have recently reported a consanguineous family with autosomal recessively inherited neonatal diabetes (Fig. 1) (4). In all cases, neonatal diabetes was associated with severe intrauterine growth retardation, microcephaly, and cerebellar hypoplasia/agenesis and very little subcutaneous fat was present. Affected individuals consistently had low levels of circulating C-peptide with very low to undetectable levels of insulin in the presence of severe hyperglycemia (typically >25 mmol/l) that was unresponsive to insulin infusion. Insulin autoantibodies were negative, and there was no evidence of pancreatic exocrine insufficiency. None of the affected individuals showed developmental progress post birth, all died before 4 months of age, and all had normal karyotypes.

From the 1Section of Cancer Genetics, Institute of Cancer Research, Sutton, U.K.; and 2North West Thames Regional Genetics Service, Kennedy-Galton Centre, North West London Hospitals NHS Trust, Harrow, U.K.

Address correspondence and reprint requests to Dr. Richard S Houlston, Section of Cancer Genetics, Institute of Cancer Research, Sutton, SM2 5NG U.K. E-mail: richard.houlston@icr.ac.uk.

Received for publication 15 May 2003 and accepted in revised form 11 July 2003.

LOD, logarithm of odds; MODY, maturity-onset diabetes of the young; PDX-1, pancreas duodenum homeobox-1; SNP, single nucleotide polymorphism; UPD6, uniparental isodisomy of chromosome 6.

© 2003 by the American Diabetes Association.
β-cells has been reported in association with UPD6 in a patient with methylmalonic acidemia (11). However, permanent diabetes has not been described in association with UPD6.

Defects in pancreatic organogenesis (isolated absence of islet cells and pancreatic hypoplasia or agenesis) have been implicated in patients exhibiting neonatal diabetes (12,13). The pancreas duodenum homeobox-1 (PDX-1) gene on 13q12.1 is critical for pancreatic development during embryogenesis (14) and is a key factor in the regulation of many important pancreatic genes, including insulin and glucokinase (15). Disruption of PDX-1 has been shown to cause pancreatic agenesis and neonatal diabetes (16). Heterozygosity for PDX-1 (MIM 125851), and MODY4 (MIM 158215) subunit (CACNB2) represent plausible candidates on the basis of either pancreatic expression in humans or mice and implied biological function of the expressed protein (17).

RESEARCH DESIGN AND METHODS

Samples. Genomic DNA was isolated using standard procedures. Informed consent was obtained from all subjects who participated in the study in accordance with local North West Regional Genetics Service ethical guidelines. Consent for autopsies was not granted.

SNP genotyping. A genomewide search was undertaken using an early access version of the GeneChip Mapping 10K Xba Array containing 10,044 SNP markers. The median intermarker distance was 105 kb, and the mean heterozygosity of markers was 0.39. On the basis of the density and informativity of SNP markers on the array, we estimate that such an analysis equates to an approximate 2.5-Mb microsatellite search. SNP genotypes were obtained by following the Affymetrix protocol for the GeneChip Mapping 10K Xba Array. Briefly, 250 ng of genomic DNA was digested per sample with the restriction endonuclease XbaI for 2.5 h. Digested DNA was mixed with Xba adapters and ligated using T4 DNA ligase for 2.5 h. Ligated DNA was added to four separate PCRs, cycled, pooled, and purified to remove unincorporated ddNTPs. The purified PCR products were then fragmentd and labeled with biotin-ddATP. Biotin-labeled DNA fragments were hybridized to the mapping 10K array 130 chip for 18 h in a standard Affymetrix 640 hybridization oven. After hybridization, arrays were washed, stained, and scanned using an Affymetrix Fluidics Station F400 with images obtained by use of the Affymetrix GeneArray scanner 2500. Affymetrix MicroArray Suite 5.0 software was used to obtain raw microarray feature intensities (raw allele scores [RAS]). Raw allele scores were processed using Affymetrix Genotyping Tools software package to derive SNP genotypes.

Statistical analysis. A search for regions of autozygosity was undertaken using the statistical package STATA version 7.0 (Stata). Whole-genome interrogation was performed by identifying only markers homozygous in the three affected individuals, heterozygous in the four parents, and heterozygous or homozygous for the alternative allele in the unaffected sibs. Further investigation of markers fitting the search criteria was undertaken by defining the length of surrounding homozygosity in the affected individuals and
heterozygosity in their parents. Multipoint linkage analysis assuming a fully penetrant autosomal recessive mode of inheritance was undertaken using the HOMOZ/MAPMAKER program (5). Equal allele frequencies for each marker and a population disease gene frequency of 0.001 were used to calculate the maximum LOD score. Statistical support for linkage was evaluated over a range of marker allele frequencies. The map order and distances between SNP markers was based on the UCSC Genome Browser (November 2002).

ACKNOWLEDGEMENTS

This work was supported by the Institute of Cancer Research.

The authors thank Michelle Bigham of Affymetrix for technical assistance and the families and physicians for their cooperation.

REFERENCES