

Brief Genetics Report

A Novel Gene for Neonatal Diabetes Maps to Chromosome 10p12.1-p13

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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.

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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.

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To identify the disease locus we carried out a genome-wide linkage search using an early access version of the GeneChip Mapping 10K Xba Array. Three affected (V:2, V:6, and V:7) and six unaffected (IV:3, IV:4, IV:8, IV:9, V:3, and V:4) family members were genotyped. We assayed one of the individuals twice (V:3) to provide an assessment of the concordance rates between the resultant genotyping calls. The average genotype call rate obtained from the 10 samples was 92.7% (range 86.7–95.5), providing data on 9.3K genotypes per individual. The concordance rate between the genotypes obtained from the repeated sample was 99.7%. Only 61 (0.06%) of the markers displayed identifiable non-Mendelian transmission and neither of the two males typed were assigned a heterozygous state for any of the 178 single nucleotide polymorphisms (SNPs) mapping to the X chromosome. A region of shared homozygosity on chromosome 10p13-p12.1 was identified in the three affected individuals. Haplotype analysis excluded other possible homozygosity-by-descent regions and identified an 8.5-Mb interval encompassing 26 informative SNPs (defined by dbSNPs rs1398431 and rs2149636). None of the unaffected family members were homozygous for the disease haplotype. Multipoint linkage analysis was undertaken using the HOMOZ/MAPMAKER program (5) assuming a fully penetrant autosomal recessive mode of inheritance, a disease allele frequency of 0.001, and equal allele frequencies of markers. The multipoint logarithm of odds (LOD) score across the 8.5-Mb region of linkage on 10p13-p12.1 was 3.25.

Neonatal diabetes differs from classical childhood type 1 diabetes because the etiology is unlikely to be autoimmune and its course is highly variable (2). Some newborns have permanent diabetes, but others have transient diabetes with lasting remissions, which occur at a median age of 3 months. Multiple studies have shown that transient diabetes predisposes one to type 2 diabetes later in life (2,6). Permanent diabetes is recognized as being associated with Wolcott-Rallison syndrome (autosomal recessively inherited neonatal diabetes, osteopenia, and epiphyseal dysplasia) caused by mutation in the eukaryotic-translation initiation factor 2- α kinase 3 (*EIF2AK3*) gene on 2p12 (7), X-linked phosphoribosyl-pyrophosphate synthetase (*PRPS1*) hyperactivity (8), and homozygous missense mutations in the glucokinase (*GCK*) gene (9) on 7p15-p13. Uniparental isodisomy of chromosome 6 (UPD6) and an unbalanced duplication of paternal chromosome 6 (specifically 6q22–24) have both been reported to cause transient diabetes (10). Congenital absence of

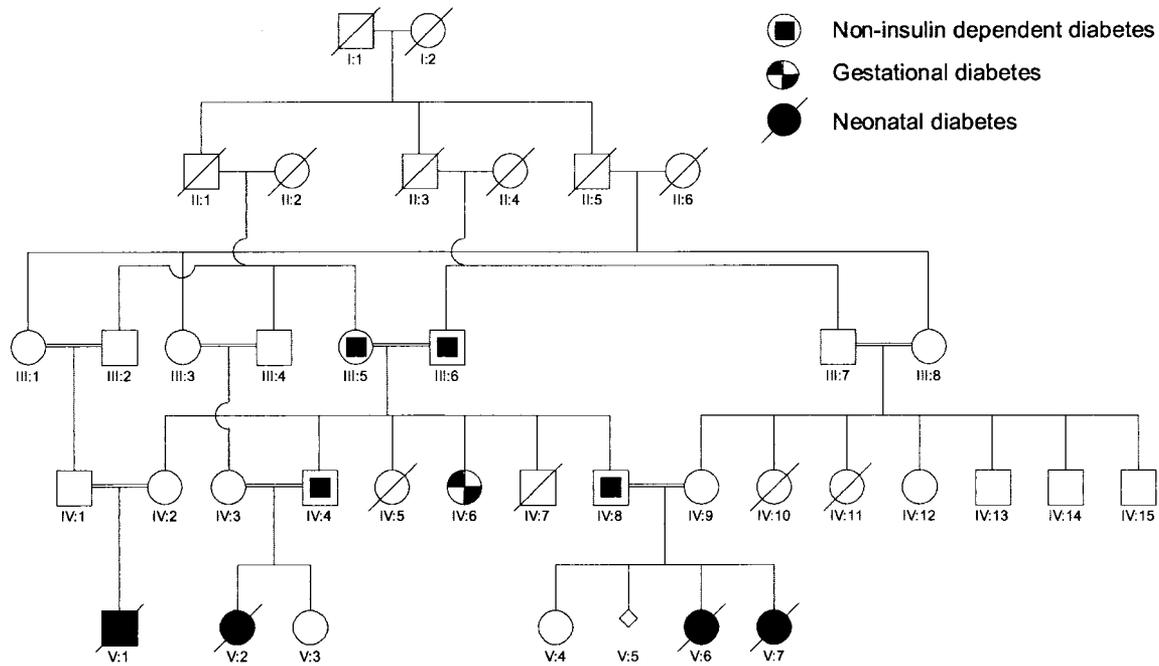


FIG. 1. Pedigree of the consanguineous family with autosomal recessively inherited neonatal diabetes.

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 *GCK* and *PDX-1* mutations predispose one to maturity-onset diabetes of the young (*MODY*), *MODY2* (MIM 125851), and *MODY4* (MIM 606392), respectively. It is likely that chromosome 10p12.1-p13 harbors another *MODY* or type 2 diabetic gene because there is a strong family history of type 2 diabetes in the absence of obesity in the family we analyzed. III:5 was diagnosed at 38, III:6 at 74, IV:4 at 34, and IV:8 at 37 years of age (Fig. 1). In addition, IV:6 had been diagnosed with gestational diabetes.

Syntenic regions in mouse, which harbor the *ad* and *fi* mouse syndromes, HSA10p11.2–12.1 and 10p13, respectively, map to our region of linkage. *Ad* defines an adult obesity and diabetes locus, whereas *fi* defines the mouse cerebellar syndrome, *fidget*. Interestingly, in addition to neonatal diabetes, the affected individuals in our family had cerebellar hypoplasia/agenesis. This suggests that the locus we have identified may be involved in neuronal development as well as diabetes.

Although we cannot exclude a contiguous-gene syndrome, any such deletion would have to be <300 Kb in size, as genotype signatures were obtained from all 37 markers mapping to the region of linkage. Forty-six transcripts map to the 8.5-Mb region of linkage (University of California, Santa

Cruz [UCSC] Genome browser, <http://genome.ucsc.edu>, release November 2002). Of these, 31 are predicted or hypothetical genes with little or no associated information regarding their biological function. At least two genes—phosphatidylinositol 4-phosphate 5-kinase, type II, α (*PIP5K2A*) and calcium channel, voltage-dependent, β -2 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 *Xba*I 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 fragmented and labeled with biotin-ddATP. Biotin-labeled DNA fragments were hybridized to the mapping 10K array 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).

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REFERENCES

- Gentz JC, Cornblath M: Transient diabetes of the newborn. *Adv Pediatr* 16:345–363, 1969
- Shield JP, Gardner RJ, Wadsworth EJ, Whiteford ML, James RS, Robinson DO, Baum JD, Temple IK: Aetiopathology and genetic basis of neonatal diabetes. *Arch Dis Child Fetal Neonatal Ed* 76:F39–F42, 1997
- von Muhlen Dahl KE, Herkenhoff H: Long-term course of neonatal diabetes. *N Engl J Med* 333:704–708, 1995
- Hoveyda N, Shield JP, Garrett C, Chong WK, Beardsall K, Bentsi-Enchill E, Mallya H, Thompson MH: Neonatal diabetes mellitus and cerebellar hypoplasia/agenesis: report of a new recessive syndrome. *J Med Genet* 36:700–704, 1999
- Kruglyak L, Daly MJ, Lander ES: Rapid multipoint linkage analysis of recessive traits in nuclear families, including homozygosity mapping. *Am J Hum Genet* 56:519–527, 1995
- Shield JP, Baum JD: Is transient neonatal diabetes a risk factor for diabetes in later life? *Lancet* 341:693, 1993
- Delepine M, Nicolino M, Barrett T, Golamaully M, Lathrop GM, Julier C, EIF2AK3, encoding translation initiation factor 2-alpha kinase 3, is mutated in patients with Wolcott-Rallison syndrome. *Nat Genet* 25:406–409, 2000
- Christen HJ, Hanefeld F, Duley JA, Simmonds HA: Distinct neurological syndrome in two brothers with hyperuricaemia. *Lancet* 340:1167–1168, 1992
- Njolstad PR, Sovik O, Cuesta-Munoz A, Bjorkhaug L, Massa O, Barbetti F, Undlien DE, Shiota C, Magnuson MA, Molven A, Matschinsky FM, Bell GI: Neonatal diabetes mellitus due to complete glucokinase deficiency. *N Engl J Med* 344:1588–1592, 2001
- Temple IK, Gardner RJ, Robinson DO, Kibirige MS, Ferguson AW, Baum JD, Barber JC, James RS, Shield JP: Further evidence for an imprinted gene for neonatal diabetes localised to chromosome 6q22–q23. *Hum Mol Genet* 5:1117–1121, 1996
- Abramowicz MJ, Andrien M, Dupont E, Dorchy H, Parma J, Duprez L, Ledley FD, Courtens W, Vamos E: Isodisomy of chromosome 6 in a newborn with methylmalonic acidemia and agenesis of pancreatic beta cells causing diabetes mellitus. *J Clin Invest* 94:418–421, 1994
- Wright NM, Metzger DL, Borowitz SM, Clarke WL: Permanent neonatal diabetes mellitus and pancreatic exocrine insufficiency resulting from congenital pancreatic agenesis. *Am J Dis Child* 147:607–609, 1993
- Roberts J, Searle J: Neonatal diabetes mellitus associated with severe diarrhea, hyperimmunoglobulin E syndrome, and absence of islets of Langerhans. *Pediatr Pathol Lab Med* 15:477–483, 1995
- Jonsson J, Carlsson L, Edlund T, Edlund H: Insulin-promoter-factor 1 is required for pancreas development in mice. *Nature* 371:606–609, 1994
- Hui H, Perfetti R: Pancreas duodenum homeobox-1 regulates pancreas development during embryogenesis and islet cell function in adulthood. *Eur J Endocrinol* 146:129–141, 2002
- Stoffers DA, Zinkin NT, Stanojevic V, Clarke WL, Habener JF: Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene coding sequence. *Nat Genet* 15:106–110, 1997
- Zhang X, Majerus PW: Phosphatidylinositol signalling reactions. *Semin Cell Dev Biol* 9:153–160, 1998