Diabetologia (2004) 47:2160–2167
DOI 10.1007/s00125-004-1576-3

Diabetologia

Neonatal diabetes, with hypoplastic pancreas, intestinal atresia and gall bladder hypoplasia: search for the aetiology of a new autosomal recessive syndrome

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Abstract

Aims/hypothesis. Neonatal diabetes is a rare disease with several identified molecular aetiologies. Despite associations with other malformations, neonatal diabetes with intestinal and biliary anomalies has not been described. The current study aims to describe a new syndrome, and to examine a possible link with one of three genes known to cause neonatal diabetes.
Methods. Five clinical cases are described. Immunohistochemical staining for pancreatic islet hormones was performed on three of the infants. DNA from one infant was analysed for abnormalities of the *PLAGL-1* (*ZAC*), glucokinase and *PDX-1* (*IPF-1*) genes.
Results. Five infants (two sibling pairs from two families, and an isolated case) presented with neonatal diabetes, hypoplastic or annular pancreas, jejunal atresia, duodenal atresia and gall bladder aplasia or hypoaplasia. One sibling pair was born to consanguineous parents. One patient with a milder form is surviving free of insulin. Four children died in the first year of life despite aggressive medical management. Pancreatic immunohistochemistry revealed few scattered chromogranin-A-positive cell clusters but complete absence of insulin, glucagon and somatostatin. Exocrine histology was variable. In one case from the consanguineous family, molecular analysis showed no duplication or uniparental isodisomy of *PLAGL-1* at 6q24, no contiguous gene deletion involving the glucokinase gene, and no mutation in the coding sequences or splice sites of *PDX-1*.
Conclusions/interpretation. This combination of multiple congenital abnormalities has not been previously described and probably represents a new autosomal recessive syndrome involving a genetic abnormality that interferes with normal islet development and whose aetiology is as yet unknown.

Keywords Duodenal atresia · Gall bladder hypoplasia · Glucokinase · Neonatal diabetes · Pancreatic hypoplasia · PDX-1 · PLAGL-1 · Syndrome.

Received: 19 April 2004 / Accepted: 31 July 2004
Published online: 8 December 2004

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Abbreviations: HIDA scan, hydroxyiminodiacetic acid scan · IPEX syndrome, immunodysregulation, polyendocrinopathy and enteropathy, X-linked syndrome · IUGR, intrauterine growth restriction · PNDM, permanent neonatal diabetes mellitus · TNDM, transient neonatal diabetes mellitus · TPN, total parenteral nutrition · UPD6pat, paternal uniparental disomy of chromosome 6

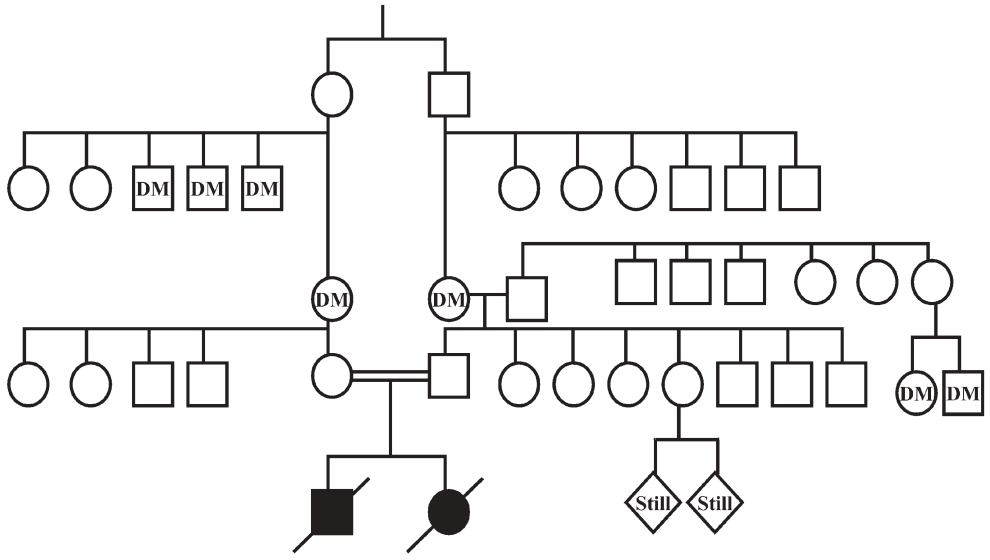

Fig. 1. Pedigree of Family 1. Parents of the two affected siblings were second cousins. There was a significant family history of type 2 diabetes (DM). The cause of two stillbirths (Still) in one paternal aunt was unknown. In this large family, there were no other known affected children

Introduction

Neonatal diabetes is a rare condition (1/500,000) [1]. The infants are usually born with intrauterine growth restriction (IUGR) and present within the first month of life with hyperglycaemia requiring insulin. Neonatal diabetes can either be transient (TNDM) or permanent (PNDM). Transient forms account for more than 50% of cases and usually resolve by 6 months of age [1, 2]. TNDM was initially linked to chromosome 6 from investigation of a child with neonatal diabetes and methylmalonic acidaemia [3]. Further analysis of this child revealed that she inherited two copies of the mutant allele from her father. In this case it was found that the neonatal diabetes resulted from an imprinted region of chromosome 6. Subsequent analysis of patients with TNDM has found that most cases (60–80%) [2, 4] can be linked to double dosage of a paternally expressed gene caused either by paternal uniparental disomy of chromosome 6 (UPD6pat), duplication of 6q24, or methylation abnormalities at 6q24 [4, 5].

PNDM is much more heterogeneous in its aetiology. Homozygosity for genes that cause MODY has been associated with several cases of neonatal diabetes. These genes have been implicated in MODY when haploinsufficiency pertains, and it is self-evident that homozygosity for these mutations may result in a more severe phenotype. Glucokinase is a key regulatory enzyme in humans that couples glucose sensing to insulin secretion. In the heterozygous individual, mutation of glucokinase can cause MODY 2 [6]. Njolstad and co-workers [7] reported two patients with PNDM who had a complete deficiency of glucokinase activity. In addition to neonatal diabetes, one of these children had total situs inversus. It has subsequently been established that glucokinase deficiency is not a common cause of PNDM [8, 9].

Another interesting case was reported by Stoffers et al. [10]. This child presented with exocrine and endocrine pancreatic deficiency and was found to have no pancreas on the ultrasound image. Its family history was positive for MODY and homozygosity for a mutation in *PDX-1* was found for the index case. *PDX-1* is an important regulatory gene involved in early pancreatic and intestinal organogenesis. Pancreatic agenesis has only been documented eight times in the world literature to date and therefore is not a common cause of PNDM.

In this study we present a case report of five children with neonatal-onset diabetes mellitus and multiple congenital abnormalities in three unrelated families. We also report the findings of our molecular analysis of *ZAC* (*PLAGL-1*), glucokinase and *PDX-1* in one of the two patients in the consanguineous family.

Subjects and methods

Family 1

Cases 1 and 2 were born to Pakistani parents who were second cousins. The family history was positive for two stillbirths born to a paternal aunt, and for type 2 diabetes in both grandmothers and more distant relatives (Fig. 1). The parents had no symptoms of diabetes and were not tested. The mother was not diagnosed with gestational diabetes during either of her pregnancies.

Case 1. This baby boy was born to a 29-year-old primigravida. Antenatal ultrasound at 24 weeks detected duodenal atresia. Amniocentesis revealed a normal 46 XY karyotype. Weight at birth, induced at 36 2/7 weeks for poor fetal growth, was 1540 g (<3%) and length was 43.5 cm (<3%). The child was not dysmorphic. On day 1 of life, the boy developed severe hy-

perglycaemia (40 mmol/l) and was started on i.v. insulin. Insulin and C-peptide levels were low in the face of hyperglycaemia. An echocardiogram revealed a patent foramen ovale with left to right shunt and minimal tricuspid regurgitation. Laparotomy at 3 days of age revealed a duodenal atresia and jejunal atresia. No gall bladder was seen at operation. He returned to surgery 1 month later for stenosis at the jejunal anastomosis. The postoperative course was complicated by total parenteral nutrition (TPN) cholestasis and severe malnutrition. A hydroxyiminodiacetic acid (HIDA) scan showed no liver excretion. Enteral feeding was never fully tolerated. This was thought to be secondary to malabsorption or villous atrophy. It was not improved with pancreatic enzyme or bile acid replacement therapy. Portal hypertension and oesophageal varices developed. The child developed disseminated candidaemia with meningoencephalitis. Immunological work-up revealed no abnormalities. Throughout this time, the boy remained insulin-dependent, requiring 0.3–0.8 units·kg^{-1}·day^{-1}. An MRI done during an acute deterioration revealed meningoencephalitis but no developmental abnormalities. Cystic fibrosis mutation analysis was negative (35 mutations). An ophthalmological examination was normal. Metabolic work-up included normal long-chain fatty acid profile, normal urine organic acids, and normal serum amino acids. The baby died at 158 days with multiple organ failure. The parents refused permission for an autopsy.

Case 2. The sister of Case 1 was noted to have dilated loops of the bowel in utero at 20 weeks gestation. Ultrasound at 24 weeks suggested this might be duodenal atresia. Amniocentesis revealed a normal 46 XX karyotype. Delivery was induced at 34 weeks due to poor fetal growth. Birthweight was 1310 g (<3%), length 40 cm (<3%) and head circumference 29 cm (<3%). Initial examination revealed a non-dysmorphic neonate with little subcutaneous fat and distended abdomen. Surgery at day 1 of life revealed distal duodenal atresia and type IIIA jejunal atresia. An annular pancreas and absence of the gall bladder were also noted. Diabetes was diagnosed at 39 hours of life with a glucose concentration of 16.9 mmol/l. Insulin and C-peptide levels were low. This child had a very similar clinical course to her sibling. A HIDA scan showed intrahepatic cholestasis with most activity remaining in the liver after 24 hours. Cholestasis progressed leading to hepatosplenomegaly and oesophageal varices. Thyroid function tests revealed normal TSH values in the face of low levels of free thyroxine, which raised the possibility of central hypothyroidism, and the child was started on L-thyroxine. A subsequent ACTH stimulation test was normal. Full enteral nutrition was never attained and was secondary to malabsorption, which did not improve with elemental formulas or addition of pancreatic enzymes. A biopsy of the liver revealed hepato-canalicular cholestasis with ductular proliferation consistent with TPN cholestasis and/or extrahepatic biliary obstruction. Duodenal endoscopic biopsies showed partial villous atrophy. Cystic fibrosis mutation analysis was negative for 35 mutations. An immunological work-up was normal. The baby died at 194 days of multi-organ failure; she weighed 6.46 kg at death. The parents consented to an autopsy that was limited to biopsies of liver, pancreas and duodenum. A sample was obtained for fibroblast culture and a portion of the liver biopsy was flash frozen for metabolic studies. Histologically, the exocrine pancreas appeared unremarkable except for mild peri-ductular inflammation. Sections from formalin-fixed, paraffin-embedded pancreatic tissue were also prepared for immunohistochemical analysis using standard methods of detection (Ventana system) for the following antibodies: chromogranin A (Vector/Novocastra; dilution 1:200), insulin (Dako; dilution 1:200), glucagon (Vector/Novocastra; dilution 1:100) and somatostatin (Dako; dilution 1:500). Our normal control subject was an age-matched infant who died from sudden infant death syndrome. Immunostaining for chromogranin A revealed occasional positive cells, individually or in small clusters but no well-formed islets (Fig. 2a). There were no cells positive for insulin, glucagon or somatostatin (Fig. 2b, c and d respectively).

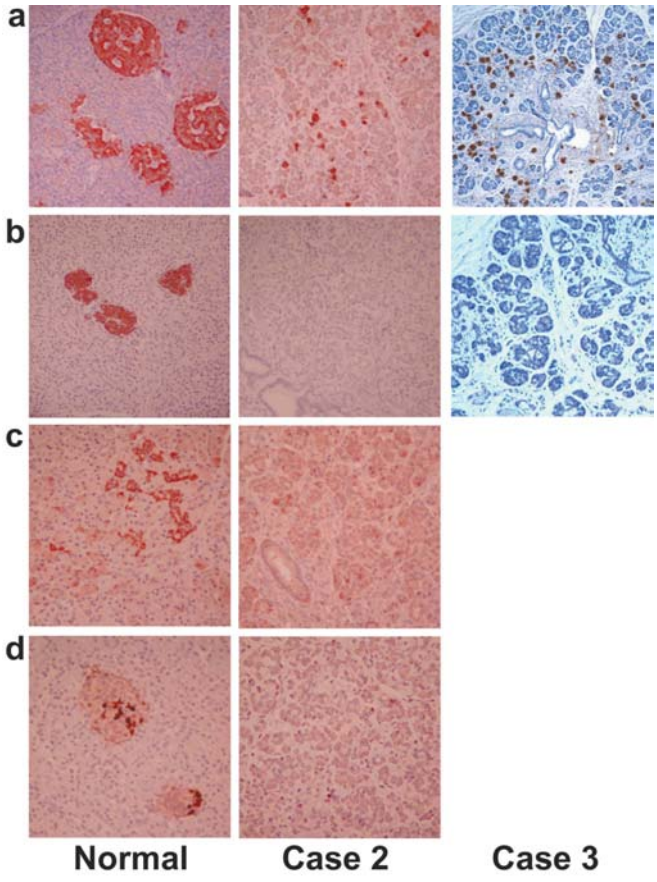

Fig. 2. Immunohistochemical staining of pancreas at autopsy for Case 2 and Case 3, compared to a normal control subject. **a.** Staining for chromogranin A. The normal control shows neuroendocrine cells organised in islets. Case 2 and Case 3 have small aggregates of neuroendocrine cells with no well-organised islet structures. Insulin staining (**b**) was present in the islets of the normal control subject, but was completely absent in Cases 2 and 3. Glucagon (**c**) and somatostatin (**d**) staining showed the expected distribution in the islets of the control subject, and no staining in Case 2. Islet structures were well preserved in the control subject, although autolysis of acini was more severe there than in the two cases. Absence of islets in the cases was not due to autolysis. Case 3 (**b**) also illustrates dilated intralobular ducts, reduced amount of exocrine glands and increased fibrous tissue

Family 2

Cases 3 and 4 were born to non-consanguineous Asian parents. There was also an unaffected, healthy brother, who was second in birth order. The maternal grandfather of the affected children had type 2 diabetes. There was no history of diabetes in other members of the extended family. The father had no symptoms of diabetes and was not tested. The mother had insulin-treated gestational diabetes during her second pregnancy,

and insulin-treated diabetes diagnosed during the 18th week of her third pregnancy with Case 4.

Case 3. This baby girl was born at 40 weeks after an unremarkable pregnancy. Delivery was uncomplicated and birthweight was 1456 g (<3%), head circumference was 30 cm (<3%) and length was 41 cm (<3%). There were no significant dysmorphic features. Insulin treatment for hyperglycaemia was initiated on day 2 of life, at which time jejunal atresia and duodenal atresia were repaired. Postoperatively, there was poor weight gain, conjugated hyperbilirubinaemia and wide swings in her blood glucose levels. Eventually, she developed septicaemia, pancytopenia, renal, liver and respiratory failure. On day 24 of life, supportive therapy was withdrawn. Head imaging was unremarkable and the karyotype was normal 46 XX. Autopsy showed repaired jejunal and pyloric atresias with intact anastomoses, annular pancreas and absent gall bladder. The post-mortem cholangiogram showed two ducts draining bile separately into pylorus and duodenum. Histological examination of the pancreas showed severe atrophy of the exocrine component with dilatation and plugging of the ducts reminiscent of cystic fibrosis. In addition, there was fibrosis of exocrine tissue, which may have been the cause of the malabsorption. The endocrine pancreas was assessed using the Ventana automated immunohistochemistry system and the following antibodies: chromogranin A (rabbit polyclonal Ab, Dako, 1:200 dilution), insulin (monoclonal Ab/Novocastra, 1:400 dilution), glucagon (rabbit polyclonal Ab, Zymed, 1:200 dilution), and somatostatin (rabbit polyclonal Ab, Dako, 1:200 dilution). Our control pancreas was from a term neonate who died of perinatal asphyxia (not shown). Immunostaining demonstrated small clusters of chromogranin-A-positive cells (Fig. 2a). However, there were no insulin-positive cells (Fig. 2b). There were no cells positive for glucagon or somatostatin (data not shown).

Case 4. The brother of Case 3 was born at 38 weeks by elective Caesarian section. The pregnancy was complicated by diabetes requiring insulin. Ultrasound at 18 weeks was unremarkable. Serial ultrasounds in the third trimester diagnosed intrauterine growth restriction and intestinal atresia. Delivery was uncomplicated and birthweight was 1610 g (<3%), head circumference was 32.5 cm (<3%) and length was 43 cm (<3%). Again there were no dysmorphic features. Starting from day 1 of life, this boy required insulin for hyperglycaemia and developed a coagulopathy. In the 1st week, duodenal and jejunal atresia were repaired. Intra-operatively gall bladder agenesis was also noted. Postoperatively, this boy had wide swings in blood glucose levels, and low C-peptide levels indicating pancreatic endocrine insufficiency. Over the next 4 months, there was poor weight gain, conjugated hyperbilirubinaemia, persistent insulin requirements and watery colourless stools. Liver biopsy showed a paucity of intralobular bile ducts. Death was from fulminant liver failure. Head imaging was unremarkable and the karyotype was normal 46 XY. Autopsy showed repaired jejunal and duodenal atresias, short pancreas and an extremely hypoplastic gall bladder. Pancreatic histology and the pattern of immunostaining for chromogranin A, insulin, glucagon and somatostatin were identical to Case 3 (data not shown).

Isolated case

Case 5. This baby girl was born small for gestational age (birthweight 2295 g) at 39 weeks to a 46-year-old mother, conceived by in vitro fertilisation with a donated egg. No family history was available. Ultrasound suggested duodenal atresia. Delivery was without difficulty. At 2 days, laparoscopy demonstrated duodenal web and malrotation; duodenal bands were lysed. Her postoperative course was complicated by airway oedema and probable sepsis. She was hyperglycaemic on 5% dextrose and required insulin briefly with resolution of hyperglycaemia. She was re-admitted at 1 1/2 months for failure to thrive, hyperbilirubinaemia and bilateral inguinal hernias. Her stools were acholic. Ultrasound failed to detect any gall bladder and the pancreas was small. Biopsy demonstrated normal intra- and extrahepatic ducts and extensive canalicular cholestasis. Again, she was hyperglycaemic and required insulin. C-peptide was low (0.30 nmol/l) and insulin was less than 14 pmol/l. Serum trypsin was also low (8 U/l). Karyotype was 46 XX and cystic fibrosis mutation analysis was negative (32 mutations). She was discharged on ursodiol and diluted ultralente insulin given as 0.4 units every 8 hours with supplemental regular insulin as needed. She had a 1-month trial of pancreatic enzyme supplementation. Irritability and feeding problems improved with small volume feeding. Insulin treatment was gradually decreased because of hypoglycaemia and was discontinued at 4 1/2 months. Her post-feeding C-peptide was still low at 5 months (0.17 nmol/l) with a normal HbA_1c of 5.1%. She gained weight steadily after her second hospitalisation and was on the 25th percentile for weight and length at 8 months with measurements of blood sugar almost all less than 8.3 mmol/l. At 1 year of age, her HbA_1c had risen to 7.5% without insulin treatment, suggesting mild but permanent diabetes.

Results

Genetic studies were undertaken only for Case 2 from Family 1 and her parents. Informed consent was obtained from the parents according to the Declaration of Helsinki. Family 2 and the parents of Case 5 were unwilling to consent to genetic studies at this point in time.

In preliminary molecular analysis of Case 2, we ruled out three of the six loci known to be associated with neonatal-onset diabetes. We did not screen for the mutations causing the Wolcott-Rallison syndrome (absence of epiphyseal dysplasia in all our cases), the IPEX syndrome (X-linked, incompatible with the pattern of inheritance in our families), or Kir6.2 mutations. Screening for uniparental disomy of chromosome 6q24 was done using the deletion/insertion polymorphism *rs3060705* which maps to the untranslated region of *PLAGL-1* (*ZAC*), the gene whose double dose is presumed to cause TNDM. PCR of this polymorphism in Case 2 (using primers 5′GTATTATCTATTTCAGGTCAGTGTG3′ and 5′GACCAGTAAAAACACTTGAACA3′) revealed two bands of equal intensity when compared with heterozygous and homozygous controls (Fig. 3). This excluded the possibility of uniparental isodisomy or duplication for chromosome 6q24 but does not rule out uniparental heterodisomy or a methylation defect. However, those are not known to be inherited, and cannot explain the other manifestations of the syndrome. The sequences flanking the glucokinase gene amplified well by PCR (us-

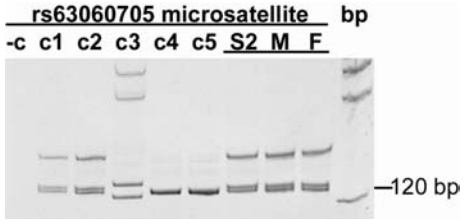

Fig. 3. *ZAC* gene 3′ UTR microsatellite ruled out paternal duplication. Case 2 (S2) and her mother (M) and father (F) have two alleles with the same allelic balance as the non-diabetic control subjects with the same alleles (c1 and c2), ruling out a duplication. The two distinct alleles in S2 also rule out uniparental isodisomy but not heterodisomy, neither of which would be likely to occur in two siblings

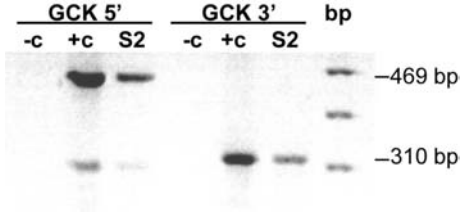

Fig. 4. Contiguous gene deletion involving *glucokinase* was ruled out by PCR amplification. PCR demonstrated the presence of a 469 bp sequence at the 5′ end of the *glucokinase* gene and a 310 bp sequence at the 3′ end of the *glucokinase* gene Case 2 (S2). Positive (+c) and negative (–c) controls are shown

ing primer pair 5′ACACTTGAAAAGTGGCCGA3′ and 5′ACCTCTTGGCAGCCTGACTT3′ for the 5′ sequence, and 5′TGCCATCTTTCTGCAGCATA3′ and 5′TCCACTCTGTGAATGCTTGA3′ for the 3′ sequence), excluding a large deletion involving this gene that could cause a contiguous-gene syndrome (Fig. 4). Detailed mutation analysis was not done, as an isolated glucokinase deficiency causing diabetes would not explain the other features of the syndrome. *PDX-1* (*IPF-1*) was by far the best candidate gene as it is expressed in the developing intestine and pancreas. Total loss of function causes pancreatic agenesis but no intestinal or liver problems [9]. To rule out a mutation involving gain of abnormal function that could explain the other abnormalities, both *PDX-1* exons and their intronic splice sites were amplified by PCR and sequenced (for exon 1, using primers 5′GAGCCTATGGTGCGGCGG3′ and 5′TGGGAGCGCTTGGAGGTAA3′; and three overlapping primer pairs for exon 2, namely 5′CTTGAAGGGGTTTGGGCTG3′ and 5′CAACTGGCACATGCGCCT3′; 5′AGGAACCACGATGAGAGGCA3′ and 5′TGG-CATCAATTTCACGGGAT3′; and 5′ACACATTGGAAGGCTCCCTAA3′ and 5′AGAAGAAAGGGGTGCAAGTT3′). No mutations were found (data not shown).

Discussion

Our five cases presented with neonatal diabetes and congenital abnormalities of the pancreas, duodenum, jejunum and biliary system associated with intrauterine growth restriction and malabsorption. Cases 1 to 4 had a severe, fatal form. Case 5 seems to have a less severe form of the same syndrome and has survived with mild diabetes mellitus, corrected duodenal web, and an absent gall bladder. Although we hypothesise a common aetiology, we cannot exclude the possibility that the three families have phenotypically similar but genetically distinct disorders affecting the same embryological pathways through mutations of different genes.

Among the four patients who died, there was variability in the degree of exocrine pancreatic insufficiency, with those in Family 1 having no response to pancreatic enzyme supplementation, and histological examinations from Case 2 showing no exocrine pancreatic pathology, whereas both children from Family 2 had abnormal exocrine histopathology. Despite this exocrine variability, the endocrine clinical and immunohistochemical findings were uniform.

Neonatal diabetes is normally an isolated finding but a few cases have been associated with congenital malformations. Stoffers and co-workers [10] reported a case of neonatal diabetes associated with pancreatic agenesis. This was attributed to homozygosity for a single nucleotide deletion in the *PDX-1* gene. Another study [11] reported siblings with neonatal diabetes and cerebellar hypoplasia. These two children also had triangular faces, small chins and low set ears. One of the infants also had optic nerve hypoplasia. An association between neonatal diabetes and macroglossia and umbilical hernia has also been reported [12, 13]. Recently, activating mutations of the gene encoding Kir6.2 subunit of the beta cell K_{ATP} channel have been identified in patients with permanent neonatal diabetes with or without dysmorphic features and neurological abnormalities [14]. A review of the literature also revealed a number of cases with similar gastrointestinal malformations to those described in our patients, but those cases had other findings in addition, e.g. tracheo-oesophageal fistula and hypospadias, and did not present with neonatal diabetes (Table 1) [15, 16, 17].

We chose to analyse genes that have been implicated in either TNDM or PNDM. TNDM results from a double dose of a gene that is normally expressed only from the paternal chromosome [2, 18, 19]. This dosage error may occur from paternal duplication, paternal UPD or methylation errors in which the normally inactivated maternal chromosome remains active. The *ZAC* gene (zinc finger protein which regulates apoptosis and cell cycle arrest) has been localised to this chromosome region. *ZAC* is known to be imprinted with exclusive paternal expression and is capable of inducing G_1 cell cycle arrest and apoptosis [20]. It is

Table 1. Clinical and pathological comparison with similar cases in the literature

	Case 1 [15]	Case 2 [15]	Case 1 [16]	Case 2 [16]	Case 1 [17]	Our Case 1	Our Case 2	Our Case 3	Our Case 4	Our Case 5
Gestational age (weeks)	40		37	39	34	36	34	40	38	39
Birthweight (g)	1350	1470	1700	1547	2150	1540	1310	1456	1610	2295
Sex	F	M	M	F	M	M	F	F	M	F
Karyotype		46XY	46XY	46XX	46XY	46XY	46XX	46XX	46XY	46XX
Consanguinity	+	+	+	+	–	+	+	–	–	–
Age at death (days)	43	110	21	81	10 months	158	194	24	4 months	Alive at 8 months
Neonatal diabetes	–	–	–	–	–	+	+	+	+	+
Oesophageal atresia/fistula	–	+	+	–	+	–	–	–	–	–
Duodenal atresia malrotation	+	+	+	+	Stenosis	+	+	Pyloric	+	Web,
Jejunal atresia						+	+	+	+	–
Hypoplastic pancreas	+	+	+	–	Annular	+	Annular	Annular	+	+
Intrahepatic biliary atresia			+	+	–	+	+		+	–
Extrahepatic biliary atresia	+	–	+	+	+			–	–	–
Gall bladder hypoplasia		+			+	+	+	+	+	+
Hypospadias	n/a	+	–	n/a	+	–	n/a	n/a	–	n/a
Cardiac	–	–	–	ASD	ASD	PFO	–	–	–	–
Central nervous system			Abnormal white matter							

+, present; –, absent; n/a, not applicable; ASD, atrial septal defect; PFO, patent foramen ovale

also a transcriptional regulator of the type 1 receptor for the pituitary adenylate cyclase activating peptide ($PACAP_1$-R) [21], which is a potent regulator of insulin expression [22].

The clinical features of TNDM do not include any of the major malformations seen in our patients, but since chromosome 6q24 has been implicated in the majority of cases of neonatal diabetes, this is an important aetiology to examine. Our patient was found to be heterozygous for *rs3060705* and densitometric analysis ruled out the possibility of paternal duplication. We cannot rule out the possibility of a methylation defect, but as other cases have not been associated with multiple congenital anomalies, it seems unlikely that the 6q24 locus is implicated in our cases.

Glucokinase is expressed in hepatocytes and beta cells where it phosphorylates glucose and is believed to be the rate-limiting step in the beta cell sensing apparatus [23]. Homozygosity for a missense mutation in the glucokinase gene has resulted in PNDM in two siblings [7]. No cases of glucokinase deficiency were associated with major malformations other than situs inversus. We could not think of a mechanism capable of explaining the features seen in our cases other than a large deletion causing a contiguous gene syndrome. The fact that we were able to amplify flanking sequences on both sides of the gene eliminated this possibility and makes it unlikely that glucokinase is involved in our cases.

PDX-1 is a gene that is central to the formation of the pancreas. It is one of the first genes expressed in the duodenal region that subsequently develops into pancreas. *PDX-1* is believed to down-regulate many genes that are characteristic of neighbouring endoderm such as *SHH*, *SOX2* and *HEX*, and it is this regional patterning that allows development of the pancreas [24]. In mice, *PDX-1* knock-outs form initial pancreatic buds but these buds do not expand [25]. In humans, there is a case report of pancreatic agenesis associated with homozygosity for a single base pair deletion. Interestingly, heterozygous mutations in the *PDX-1* gene were subsequently found to cause MODY 4 and this further underlines the importance of work-up of these rare cases for understanding more common forms of diabetes. Sequence analysis of the *PDX-1* gene revealed no mutations in the coding region or splice sites. This does not rule out upstream regulatory mutations, but the one homozygous case and the knock-out mouse demonstrated that total loss of *PDX-1* function causes complete agenesis of the exocrine as well as the endocrine pancreas, which was not the case in our patients. Since *PDX-1* is also involved in intestinal development (its role in the development of the biliary tract has not been examined), we felt that the extrapancreatic manifestations of the syndrome could be explained not by quantitative *PDX-1* deficiency, but rather by some gain-of-function mutation in the structural protein. None was found.

As our patients presented with neonatal diabetes associated with gastrointestinal malformations, it is important to analyse genes that could potentially affect the embryogenic development of the fetus. As the pathology showed abnormalities in multiple pancreatic cell lines, the defect must occur at an early stage in

embryonic development. The finding of chromogranin-A-positive cells in our patients may be of interest, since this could be a marker of precursor cells that failed to differentiate. Chromogranin A is usually localised in neurosecretory granules, yet presumably these cells lack such granules.

Our understanding of pancreatic development has resulted from extensive studies in the mouse and chick [24, 25]. Intracellular signalling pathways that are implicated in human diseases include the Notch, TGF-β and Hedgehog pathways (see review in Kim and Hebrock [25]). Future research on DNA from these patients will analyse the genes that have been determined to play a role in controlling these pathways, such as *Isl-1*, which is important for both dorsal pancreatic budding from the gut epithelium and differentiation of islet cells [26], and *Hes-1*, which prevents premature differentiation of pancreatic exocrine and endocrine cells and gut endocrine epithelium [27]. The long list of candidate genes can be pared down by homozygosity mapping in the consanguineous family, potentially narrowing the locus down to a few centimorgans.

The cases presented here represent a new autosomal recessive syndrome not previously identified. Prenatal ultrasound with findings suggestive of intrauterine growth restriction, intestinal atresia and gall bladder agenesis/hypoplasia should prompt clinicians to be vigilant for neonatal diabetes and to discuss the possibility of autosomal recessive inheritance with their patients.

Determining the aetiology is not only important for genetic counselling and prenatal diagnosis of this very rare disorder, but it is likely to reveal novel information about the genes involved in the embryogenesis of the endocrine pancreas. This will have implications for our understanding of beta cell mass generation and maintenance, as well as in the effort to develop cell-based treatments, and therefore be relevant to both common types of diabetes.

Acknowledgements. The first two authors, John Mitchell and Zubin Punthakee, contributed equally to this work. This work was supported by a grant from the Juvenile Diabetes Research Foundation to C. Polychronakos. We would like to thank Dr J.M. Laberge and J. Perrault for their involvement in the clinical evaluation of cases 1 and 2.

References

1. Von Muhlendahl KE, Herkenhoff H (1995) Long-term course of neonatal diabetes. New Engl J Med 333:704–708
2. Temple IK, Gardner RJ, Mackay DJ, Barber JC, Robinson DO, Shield JP (2000) Transient neonatal diabetes: widening the understanding of the etiopathogenesis of diabetes. Diabetes 49:1359–1366
3. Abramowicz MJ, Andrien M, Dupont E et al. (1994) 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
4. Metz C, Cave H, Bertrand AM et al. (2002) Neonatal diabetes mellitus: chromosomal analysis in transient and permanent cases. J Pediatr 141:483–489
5. Whiteford ML, Narendra A, White MP et al. (1997) Paternal uniparental disomy for chromosome 6 causes transient neonatal diabetes. J Med Genet 34:167–168
6. Njolstad PR, Cockburn BN, Bell GI, Sovik O (1998) A missense mutation, Val62Ala, in the glucokinase gene in a Norwegian family with maturity-onset diabetes of the young. Acta Paediatr 87:853–856
7. Njolstad PR, Sovik O, Cuesta-Munoz A et al. (2001) Neonatal diabetes mellitus due to complete glucokinase deficiency. New Engl J Med 344:1588–1592
8. Vaxillaire M, Samson C, Cave H, Metz C, Froguel P, Polak M (2002) Glucokinase gene mutations are not a common cause of permanent neonatal diabetes in France. Diabetologia 45:454–455
9. Gloyn AL, Ellard S, Shield JP et al. (2002) Complete glucokinase deficiency is not a common cause of permanent neonatal diabetes. Diabetologia 45:290
10. Stoffers DA, Zinkin NT, Stanojevic V, Clarke WL, Habener JF (1997) Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene coding sequence. Nat Genet 15:106–110
11. Hoveyda N, Shield JP, Garrett C et al. (1999) Neonatal diabetes mellitus and cerebellar hypoplasia/agenesis: report of a new recessive syndrome. J Med Genet 36:700–704
12. Dacou-Voutetakis C, Anagnostakis D, Xanthou M (1975) Macroglossia, transient neonatal diabetes mellitus and intrauterine growth failure: a new distinct entity? Pediatrics 55:127–131
13. Salerno M, Gasparini N, Sandomenico ML, Franzese A, Tenore A (1994) Two interesting cases of transient neonatal diabetes mellitus. J Pediatr Endocrinol 7:47–52
14. Gloyn AL, Pearson ER, Antcliff JF et al. (2004) Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. New Engl J Med 350:1838–1849
15. Martinez-Frias ML, Frias JL, Galan E, Domingo R, Paisan L, Blanco M (1992) Tracheoesophageal fistula, gastrointestinal abnormalities, hypospadias, and prenatal growth deficiency. Am J Med Genet 44:352–355
16. Anneren G, Meurling S, Lilja H, Wallander J, von Dobeln U (1998) Lethal autosomal recessive syndrome with intrauterine growth retardation, intra- and extrahepatic biliary atresia, and esophageal and duodenal atresia. Am J Med Genet 78:306–307
17. Gentile M, Fiorente P (1999) Esophageal, duodenal, rectoanal and biliary atresia, intestinal malrotation, malformed/hypoplastic pancreas, and hypospadias: further evidence of a new distinct syndrome. Am J Med Genet 87:82–83
18. Temple IK, Gardner RJ, Robinson DO et al. (1996) Further evidence for an imprinted gene for neonatal diabetes localised to chromosome 6q22-q23. Hum Mol Genet 5:1117–1121
19. Kamiya M, Judson H, Okazaki Y et al. (2000) The cell cycle control gene ZAC/PLAGL1 is imprinted—a strong candidate gene for transient neonatal diabetes. Hum Mol Genet 9:453–460
20. Spengler D, Villalba M, Hoffmann A et al. (1997) Regulation of apoptosis and cell cycle arrest by Zac1, a novel zinc finger protein expressed in the pituitary gland and the brain. EMBO J 16:2814–2825
21. Ciani E, Hoffmann A, Schmidt P, Journot L, Spengler D (1999) Induction of the PAC1-R (PACAP-type I receptor) gene by p53 and Zac. Brain Res Mol Brain Res 69:290–294

22. Yada T, Sakurada M, Nakata M, Shioda S, Yaekura K, Kikuchi M (1998) Autocrine action of PACAP in islets augments glucose-induced insulin secretion. Ann NY Acad Sci 865:451–457
23. Matschinsky F, Liang Y, Kesavan P et al. (1993) Glucokinase as pancreatic beta cell glucose sensor and diabetes gene. J Clin Invest 92:2092–2098
24. Grapin-Botton A, Majithia AR, Melton DA (2001) Key events of pancreas formation are triggered in gut endoderm by ectopic expression of pancreatic regulatory genes. Genes Dev 15:444–454
25. Kim SK, Hebrok M (2001) Intercellular signals regulating pancreas development and function. Genes Dev 15:111–127
26. Ahlgren U, Pfaff SL, Jessell TM, Edlund T, Edlund H (1997) Independent requirement for ISL1 in formation of pancreatic mesenchyme and islet cells. Nature 385:257–260
27. Jensen J, Pedersen EE, Galante P et al. (2000) Control of endodermal endocrine development by Hes-1. Nat Genet 24:36–44