# β-Cell Dysfunction in Classic Transient Neonatal Diabetes Is Characterized by Impaired Insulin Response to Glucose but Normal Response to Glucagon

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**OBJECTIVE** — To investigate β-cell function and the long-term health of four case subjects presenting with chromosome 6–associated transient neonatal diabetes (TND).

**RESEARCH DESIGN AND METHODS** — Two unrelated case subjects presenting with paternal uniparental isodisomy of chromosome 6 (UPD6) and two siblings with a paternally inherited duplication of 6q24 were studied. Three case subjects presented with neonatal diabetes that recurred at 4–17 years, while diabetes was incidentally discovered in the other case subject at 14 years of age. β-Cell function was investigated after diabetes relapse by means of an oral glucose tolerance test (OGTT), an intravenous glucose tolerance test (IVGTT), and glucagon tests. The quantitative insulin sensitivity check index (QUICKI) was calculated from fasting blood samples as an estimate of insulin sensitivity.

**RESULTS** — β-Cell function was investigated at diabetes relapse in two case subjects: the insulin response to both an OGTT and IVGTT was low, whereas the basal levels of C-peptide were normal. No evidence of insulin resistance was found. Residual β-cell function was further explored by a glucagon test in all subjects at the age of 16–28 years and was found to be normal. Final height was within the normal percentiles, whereas one case, who had been poorly controlled since puberty, presented with diabetes-related microvascular complications.

**CONCLUSIONS** — In patients with chromosome 6–associated TND, the β-cell is preserved and able to secrete insulin through the stimulatory G protein pathway while exhibiting a specific defect of insulin secretion after glucose stimulation. This form of diabetes can be managed with insulin or diet, although new therapeutic agents (glucagon-like synthetic analogs) may prove useful in the future. Lack of treatment leads to long-lasting hyperglycemia without the risk of ketoacidosis but associated with microangiopathy in adult life.

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Transient neonatal diabetes (TND) is a rare (1/400,000 live births) but well-recognized disorder manifesting in the early neonatal period with hyperglycemia, dehydration, and minimal ketosis. Most patients are full-term, but growth-retarded, infants. Apparent remission usually occurs by 3 months, and relapse of diabetes has been reported in ~50–60% of cases in late childhood or early adult life (1).

Recent progress in molecular analysis has indicated (2–5) that TND is a disease entity, distinct from permanent neonatal diabetes. Three interrelated genetic mechanisms have been ascribed to TND (6–8): paternal uniparental isodisomy of chromosome 6 (UPD6), paternal duplication of 6q24, and a methylation defect at a CpG island overlapping exon 1 of ZAC (zinc finger protein associated with apoptosis and cell cycle arrest)/HYMAI (imprinted in hydatidiform mole) (9). These imprinted genes may be implicated in the pathogenetic mechanism of diabetes, owing to their hypothetical role in insulin secretion. An inadequate insulin secretory response to glucose stimulation was found in islet cells from transgenic mice overexpressing the TND locus (G. Kelsey, personal communication). Moreover, ZAC regulates the expression of receptor 1 of the pituitary adenylate cyclase activating polypeptide, a potent insulin secretagogue and an important mediator of autocrine control of insulin secretion (10).

The few clinical studies available in these patients agree that poor control of insulin release rather than an actual inability to produce insulin may explain the disease (11). The intravenous glucose tolerance test (IVGTT) has been mainly used to explore β-cell function (12,13). In addition, little data are available on the long-term course of the disease. In a survey of 13 patients with TND with later recurrence, the longitudinal growth was not

impaired (2,4,12) and no microangiopathic complications were reported (14).

We describe data regarding β-cell function, explored by OGTT, IVGTT, and the glucagon test, and follow-up into adulthood in four case subjects presenting with chromosome 6 anomalies.

## RESEARCH DESIGN AND METHODS

— Three unrelated patients (CM, CN, and CA) with classic TND and the brother (CE) of one of them (CA) were recruited at the Department of Pediatrics, Federico II University, Naples. All patients had intrauterine growth retardation; three of whom presented with neonatal diabetes without ketonuria between 7 and 31 days of life. The remaining case subject (CE) was incidentally found to be hyperglycemic at age 14 years. The three neonatal case subjects were treated with 1–2 units of insulin until diabetes remission, which occurred at 5 weeks and 6 and 15 months of age. Diabetes relapsed, respectively, at age 17, 8, and 4 years and was treated with diet in the former and insulin in the latter two patients. Pubertal development occurred normally in all patients, apart from CM, who developed precocious puberty (breast stage 2 at age 7.0 years), but did not require any treatment since her predicted final height was appropriate for target parental height. Type 2 diabetes was diagnosed in case subject CE at 14 years of age, and an oral hypoglycemic agent was prescribed. He refused any treatment and only occasionally underwent medical assessment; he had a persistently high HbA$_{1c}$ level (>10%, normal values <6.5%). He first came to our notice at 28 years of age. Insulin therapy was started, and his HbA$_{1c}$ dropped to 7.6% after 3 months. Subsequently, he missed all of his further appointments, refusing any treatment. An adult diabetologist, who prescribed a further oral hypoglycemic agent, is now following him. His diabetes control remains very poor.

Phenotypic HLA was DR3, DR4, and DQ2 negative in all patients except CM (DR4$^+$). Routine karyotype was normal in all patients, apart from CM (47 XXX). Molecular studies on chromosome 6 revealed the presence of UPD6 in CM and CN and a submicroscopic duplication of the TND critical region of chromosome 6q24 in CA and CE (2). Their father carried the same 6q24 duplication, inheriting it from his mother. Further clinical details about CM and CN have been previously reported (2,15,16).

β-Cell function was explored at diabetes relapse and at the last review by OGTT (1.75 g/kg, maximum 75 g), IVGTT (0.5 g/kg, maximum 35 g), and/or glucagon (1 mg) stimulation. In particular, the OGTT and IVGTT were performed in patients who were not yet treated with insulin, while glucagon was used in all patients, irrespective of their insulin requirement. During the OGTT, plasma samples were taken at 0, 30, 60, 90, and 120 min for measurements of plasma glucose and serum insulin. From fasting blood samples the quantitative insulin sensitivity check index (QUICKI) was calculated (1/log fasting insulin [μU/ml] + log glucose [mg/dl]) as an estimate of insulin sensitivity (17). The IVGTT was performed by intravenous infusion of a 25% glucose solution over 2.5–3 min by manual-driven syringe and timed to ensure a steady infusion rate. Time zero is defined as the end of the infusion. Baseline samples from a separate cannula were taken 10 min before and further samples at 1, 3, 5, and 10 min after the end of glucose infusions for the determination of insulin levels. The first-phase insulin response, expressed as the sum of insulin values at 1 and 3 min, was considered pathological when it was below the first percentile of the reference values (18). The intravenous glucagon test was performed at plasma glucose levels <7 mmol/l. C-peptide was measured in the basal state and 6 min after glucagon stimulation, and the relative increase was calculated (stimulated/basal × 100, normal values 130–377%) (19).

Insulin was measured by an immunometric assay (Immulite 2000 Insulin; Diagnostic Products, Los Angeles, CA) that uses a monoclonal murine anti-insulin antibody specific for insulin, with a 8% cross-reactivity with proinsulin. The intra-assay coefficient of variation (CV) was <5.5% and the interassay CV <7.3%. C-peptide was measured by an immunometric assay (Immulite 2000 C-Peptide; Diagnostic Products) that uses a polyclonal rabbit anti–C-peptide antibody specific for C-peptide, with 17% cross-reactivity with proinsulin. The intra-assay CV was <14.1% and the interassay CV <18.6%. Informed consent of the patients and their parents was obtained before performing the diagnostic tests.
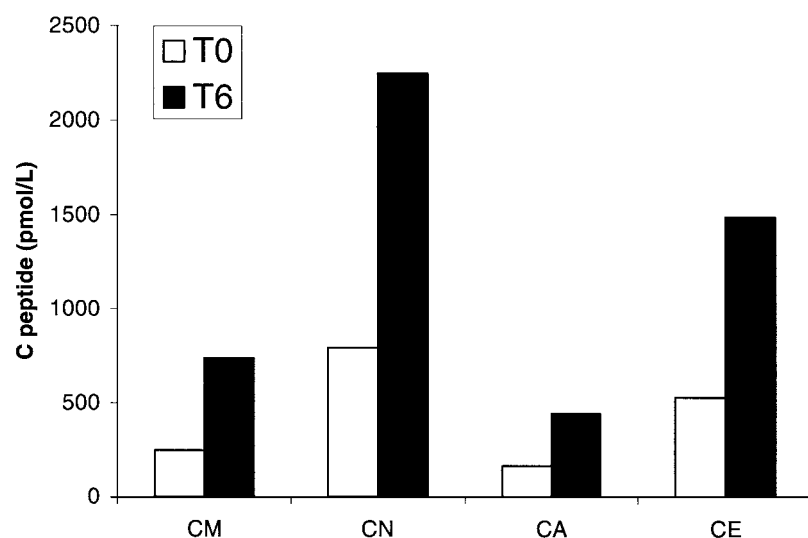
## RESULTS

— At diabetes relapse, results of OGTT and IVGTT were available in two patients (CN and CA). Both OGTTs showed a diabetic response to glucose (>11 mmol/l at 120 min), with low insulin levels (96 and 90 pmol/l, respectively). The IVGTT displayed suboptimal insulin secretion. First-phase insulin response was 264 pmol/l in CN (minimum normal value for age-matched boys, i.e., the first percentile, is 460 pmol/l) and 120 pmol/l in CA (minimum normal value for age-matched girls is 318 pmol/l). The QUICKI at this time was 0.301 and 0.331, respectively (mean value 0.359 ± 0.028 pmol/l in 32 normal control subjects aged 9.9 ± 2.3 years). Interestingly, basal C-peptide levels were normal at 364.1 and 1,026 pmol/l for CN and CA, respectively (normal range 165–993 pmol/l).

At the last review, after a duration of diabetes ranging from 1.9 to 14 years since relapse, all patients underwent a glucagon test revealing an apparently normal stimulated C-peptide response (Fig. 1), with a relative increase ranging from 268 to 297%.

Patients reached a mean final height of 169.9 ± 5.3 cm, which was higher than their sex-adjusted midparental height (163.5 ± 4.3 cm) (20), and were free of any diabetes complications, apart from CE, who attained a height lower than his target height due to the additional features of background retinopathy and persistent clinical albuminuria (albumin concentration >500 mg/l in early morning urine).

## CONCLUSIONS

— We describe β-cell function at diabetes relapse and longitudinal growth until adulthood in two unrelated case subjects with paternal UPD6 and two siblings with paternally inherited submicroscopic duplications of 6q24.

Three case subjects presented with neonatal diabetes, whereas diabetes was incidentally discovered in the other case subject at age 14 years. A neonatal presentation of diabetes is a classic finding in patients with anomalies at the TND locus, but diabetes may go unrecognized and present later. Gestational diabetes has been reported in a case subject with 6q24 duplication. Moreover, UPD6 has been detected fortuitously in people with no early history of TND (4).

**Figure 1**—*Results of the glucagon stimulation test.*

It has been postulated that the neonatal illness in TND might be due to either a defect in islet cell maturation or β-cell insulin secretory capacity. Whatever the defect, insulin levels during an IVGTT were low in at least 50% of patients during the remission phase (9,12), suggesting that the inherent defect persists during clinical remission only to become manifest at times of metabolic stress.

In keeping with this, relapse of diabetes has been reported in ~50–60% of TND case subjects (2). However this rate may increase with continued surveillance of these case subjects. Very few data are available on the β-cell function at relapse: low C-peptide levels were reported in a 9-year-old child (3). In our patients, the insulin response to both the OGTT and IVGTT was subnormal in two case subjects at diabetes relapse, whereas the basal C-peptide levels were normal. As our C-peptide assay has some cross-reactivity with proinsulin we cannot be certain that these results do not include an element of hyperproinsulinemia. However, we feel it unlikely given previous data showing normal proinsulin levels in TND patients in remission (13), with the additional fact that there was no evidence of insulin resistance in the fasting state, as indicated by the normal QUICKI.

According to the results derived from the three largest cohorts of TND patients (2,4,14), the average age at diabetes relapse is 13–16 years. This implies that puberty exerts a triggering role, owing to the increased insulin demands on a defective β-cell (21).

No studies are available on the long-term course of diabetes and the β-cell function in these patients. Treatment of diabetes after recurrence has been recently reviewed (9). Insulin is used more frequently than diet alone or oral hypoglycemic agents, and its requirement seems to be lower than that described in type 1 diabetes or may be intermittent, at least initially (2,4). There is also some evidence supporting insulin resistance (22), although a body of evidence, including that of this work, is developing against this mechanism for diabetes relapse.

We carefully monitored the insulin demands throughout pubertal development in the two girls treated with insulin. Their daily insulin dose slightly increased, between B4 and menarche, whereas HbA$_{1c}$ levels worsened. At the last review, the daily insulin dose was ~0.76 units/kg, which is similar to that required by our type 1 diabetic adolescents (0.85 ± 0.21 units/kg). CN was treated with diet, whereas CE required oral hypoglycemic agents, since he refused insulin, despite poor metabolic control. Nevertheless, he never experienced ketoacidosis, but developed microvascular complications. This is the first report of diabetes complications occurring in a TND case subject and emphasizes the importance of good diabetes management in these patients.

Further investigation of residual β-cell function has proven invaluable by offering new insights into the β-cell defect in TND. Glucagon-stimulated C-peptide response was normal in all subjects, sug-

gesting that in patients with an imprinted 6q24 anomaly, the β-cell is able to produce and secrete insulin after glucagon stimulation, while it is not able to respond to glucose stimulation either orally or intravenously. Degranulation of insulin stored in the cytoplasmic vesicles occurs along two distinct transduction pathways: *1*) glucagon acts through G protein–coupled receptors, which increase cAMP production and activate protein kinase, while *2*) glucose acts through the GLUT2 transporter, activating glucokinase and increasing calcium efflux (23). This suggests that overexpression at the TNDM locus blocks the classical glucose-stimulated insulin response.

The majority of TND infants are born small for gestational age, but their growth becomes normal within 2 years (4). Accordingly, the final height in our patients was within the normal percentiles.

In conclusion, our preliminary data suggest that in TND, due to 6q24 anomalies, the β-cell is preserved and able to secrete insulin through the stimulatory G protein pathway while exhibiting a specific defect of insulin secretion after glucose stimulation. This form of diabetes can be managed with insulin or diet, although new therapeutic agents such as glucagon-like synthetic analogs may prove useful in the future (24). Clinicians and patients should be made aware that treatment failure or poor compliance in relapse results in persisting hyperglycemia, without the risk of ketoacidosis, but associated with the risk of microangiopathy.

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