Transient Neonatal Diabetes

Widening the Understanding of the Etiopathogenesis of Diabetes

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Transient neonatal diabetes (TND) is a rare type of diabetes that presents soon after birth, resolves by 18 months, and predisposes to diabetes later in life. A total of 30 patients were ascertained and investigated for aberrations of chromosome 6. A genotype/phenotype study was also performed. Genotypically, these patients can be classified into 4 etiologic groups. Group 1 had paternal uniparental isodisomy of chromosome 6 (11 cases, including 1 set of identical twins). Group 2 had a duplication involving chromosome band 6q24, which was paternal in origin where tested (4 sporadic cases and 7 familial cases from 2 families). Group 3 consisted of 1 patient with a loss of methylation at a CpG island within the TND critical region (1 sporadic case). Group 4 had no identifiable rearrangement of chromosome 6 (7 sporadic cases). Most patients were growth retarded at birth, presented at a median age of 3 days, and recovered at a median age of 12 weeks. In group 2, 2 relatives of the TND patients who presented with type 2 diabetes and no early history of TND had inherited an identical duplication. An abnormality of chromosome 6 was identified in ~70% of sporadic TND cases and in all familial cases. No significant clinical differences were found between the 4 etiological groups. The study has broadened the clinical spectrum of TND to include type 2 diabetes presenting in later life with no neonatal presentation. The findings are consistent with an imprinted gene for diabetes mapping to 6q24, which we predict will have an important function in normal pancreatic development. *Diabetes* 49:1359–1366, 2000

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Received for publication 31 August 1999 and accepted in revised form 5 May 2000.

EST, expressed sequence tag; ID, identification number; IPF-1, insulin promoter factor 1; NHS, National Health Service; PCR, polymerase chain reaction; STS, sequence tagged site; TND, transient neonatal diabetes; UPD, uniparental isodisomy.

That susceptibility to type 2 diabetes has a genetic component is well recognized. Linkage studies in large cohorts are underway, but no novel genes have yet been identified (1). However, the study of rare types of diabetes related to single gene defects has been highly informative in our understanding of the etiopathogenesis of the diabetic condition. For example, in 1997, Stoffers et al. (2) identified a homozygous mutation of insulin promoter factor 1 (IPF-1) as the cause of pancreatic agenesis and recently showed that heterozygosity for an IPF-1 mutation predisposes to early adult-onset type 2 diabetes (3). We have taken this latter approach with a molecular analysis of transient neonatal diabetes (TND). We believe that TND likely results from a single gene defect, and, because it is associated with intrauterine growth retardation and a tendency to develop diabetes later in life, the gene may have relevance to more common types of diabetes.

TND is a rare but well-recognized type of diabetes (incidence of 1/400,000) (4). Growth-retarded infants present within the first few days of life with hyperglycemia, dehydration, and minimal ketosis. Endogenous insulin levels are low or undetectable, and exogenous insulin is usually required for a mean duration of 3 months. The condition resolves by 18 months of age, but type 2 diabetes may recur in early adulthood (4,5). TND is distinct from classic type 1 diabetes because patients do not carry the common HLA susceptibility haplotypes, and no evidence exists of autoimmune disease (6).

In 1995, Temple et al. (7) discovered that 2 cases presenting with TND had paternal uniparental isodisomy (UPD) of chromosome 6 (i.e., the inheritance of 2 chromosome homologs from one parent with no contribution from the other). A total of 5 further cases with TND and paternal UPD6 have since been reported in the literature (8–12), and another involved a child with diabetes who died at the age of 16 days (13). The association is now firmly established, although 2 cases of paternal UPD6 have been reported in older patients with no early history of TND, which cannot yet be explained (14,15). We and others have subsequently shown that several nondisomic children with TND have paternally inherited duplications within the long arm of chromosome 6 (6q23–24) (12,16–18). Evidence from one of these families suggested that inheritance of an identical duplication from the mother did not lead to TND (16). This was in keeping with the 18 cases of maternal dupli-

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cation of the region, which have been described in the literature as without neonatal diabetes (18), and likewise TND has not been reported in maternal UPD6 (19). One additional case of duplication of 6q and TND has been reported for which the origin of the duplication is unknown (20).

These observations have led to the hypothesis that overexpression of a paternally expressed allele at 6q24 causes TND with no expression of the maternally inherited allele (16,21). A critical region of duplication has been mapped by Gardner et al. (21) and lies within a 400-kb region of 6q24. A differentially methylated CpG island was found within the critical region and was methylated only on the maternally inherited chromosome 6 homolog. All patients with paternal UPD6 showed a complete lack of methylation at the site, as did 2 additional patients with TND and no other chromosome 6 abnormalities. (Only 1 of these had sufficient clinical data for inclusion in this article.) This finding suggested that methylation defects at this locus could be causative and has further strengthened the likelihood that an imprinted gene is involved. Kamiya et al. (22) reported that ZAC/PLAGL1, which is within the critical TND region and is 85 kb from the imprinted CpG island, is imprinted in fetal tissues and is thus a strong candidate gene for TND. The gene for TND is implicated in normal pancreatic function, and its discovery may further our understanding of susceptibility to more common types of diabetes.

We report an analysis of the largest cohort of TND cases studied to date and compare the phenotype with molecular findings at chromosome band 6q24.

RESEARCH DESIGN AND METHODS

Patients. Eligible patients were defined as presenting at >35 weeks of gestation with persistent hyperglycemia within the first 6 weeks of life and recovery by 18 months. The main exclusion criteria were prematurity and persistent diabetes. We recruited patients through the British Paediatric Association Surveillance Unit, clinical geneticists, neonatologists, pediatric endocrinologists, the British Diabetic Association, and authors of TND reports during the last 20 years. All referring clinicians were asked to complete a clinical questionnaire regarding birth history and subsequent progress of the child. Blood samples were requested for cytogenetic and molecular analysis.

Genetic investigation. A karyotype was determined when possible for each patient using standard techniques. A modification of the method of Pinkel et al. (23) was used for chromosome painting with a whole-chromosome paint for chromosome 6 (Cambio). DNA was extracted from blood samples of the patients and (when possible) their parents using a salt precipitation method (24).

To test for uniparental disomy of chromosome 6, the DNA samples were analyzed using polymorphic microsatellite repeats (Généthon or GDB) along the length of chromosome 6 (Table 1). The DNA was amplified in a Perkin-Elmer (Norwalk, CT) 9600 programmable heating block according to the protocol of Hudson et al. (25).

Patients who did not have uniparental disomy or a visible duplication of chromosome 6 were analyzed by fluorescent quantitative polymerase chain reaction (PCR) (21,26–28). Eight sets of primers from within the TND critical region were used to amplify nonpolymorphic unique sequences (sequence tagged sites [STSs] or expressed sequence tags [ESTs]). Primer sequences were obtained from the Sanger Centre's (Cambridge, U.K.) chromosome 6 database (http://www.sanger.ac.uk/hgp/chr6/) and were all located between stSG1437 and stSG27894 (21) (Fig. 1). These primers and a control primer pair from chromosome 5q21 were manufactured by MWG Biotech. A fluorophore was attached to the 5′ end of 1 primer in each pair. A total of 100 ng DNA was amplified by multiplexing a primer set from within the TND critical region with the control primer set. Amplification was carried out in a Perkin-Elmer 9600 programmable heating block using the following conditions: 95°C for 15 min followed by 19 cycles of 94°C for 45 s, 52–57°C (depending on the annealing temperature required by a particular primer set) for 45 s, and 72°C for 1 min. This was followed by 7 min at 72° and 1 h at 60°C. A total of 19 cycles were performed to maintain amplification within the linear range. PCR products were separated by polyacrylamide gel electrophoresis using an ABI 377 automated sequencer. The peak height of each allele was quantified using Genotyper software, and the ratio of test:control was calculated to yield a relative measurement of sequence copy number in each individual (Fig. 2). Control samples with known duplications and without duplications of the region were included in each experiment. Peak height ratio means were calculated for both sets of control subjects along with the 95% CIs and SDs. Ratios obtained from the patient samples fell within the 95% CIs of either the duplicated or control samples. Table 2 shows the results for the proband of each family with a submicroscopic duplication using 1 of the critical markers. Each patient was tested with each marker at least twice in different experiments. This method has been further substantiated as in 8 out of 9 of the submicroscopic duplications that were identified in TND probands, samples from other family members were available, and an identical duplication was demonstrated in 1 or more relatives.

After the identification of a CpG island with a methylation imprint, the methylation status of the patient DNA was determined. DNA was digested with isoschizomers *Hpa*II and *Msp*I before amplification by PCR using primers spanning CCGG sites (21).

TABLE 1
Markers used in the diagnosis of UPD6

Markers	Chromosome location	Heterozygosity (%)
D6S260	6p23–p24	84
D6S276	6p21.3–p22	83
Tumor necrosis factor-α	6p21	27
D6S286	6q14.3–q15	78
D6S292	6q21–q23.3	83
D6S262	6q21–q22.1	82
D6S314	6q13.6–q27	80
D6S308	6q23–q24	75
D6S310	6q23–q24	79

RESULTS

Genotype analysis. We ascertained 30 patients who fulfilled the criteria. Of these, 23 were sporadic cases (including 1 set of identical twins), and 7 were familial (3 from 1 family [29] and 4 from the other [30,31]). The clinical and molecular details are shown in Table 3.

We were able to divide the patients into 4 etiological groups. Group 1 consisted of 11 patients from 10 families (including 1 set of identical twins) (Table 3, identification number [ID] 1–11) who were shown to have paternal isodisomy of chromosome 6. In group 2, 11 patients were identified with a duplication of part of chromosome 6q. These included all 7 familial cases (families K and L) and 4 sporadic cases (Table 3, ID 19–22). Only 2 were cytogenetically visible (Table 3, ID 21 and 22) (16,17), and 9 were identified using quantitative PCR of ESTs and STSs from the region (Fig. 1, ID 12–20). Determining the parental origin in 9 of the duplication cases (visible and submicroscopic) was possible by testing both parents using the same method as that used for the probands. In all cases, an identical duplication was demonstrated in the father. In 2 cases (both adults, ID 12 and 20), testing was not possible because parental samples were not available. Group 3 consisted of 1 sporadic patient with a methylation mutation and no other evidence of a chromosome 6 rearrangement. Group 4 (all sporadic cases) consisted of the remaining 7 patients with no identified abnormality of chromosome 6.

Phenotype analysis. Clinical findings were compared in the 4 etiological groups. χ^2 analysis for sex, gestation, birth weight, presentation by 7 days, recovery by 2 months, and subsequent recurrence of diabetes showed no significant differences between the groups (data not shown). The numbers of

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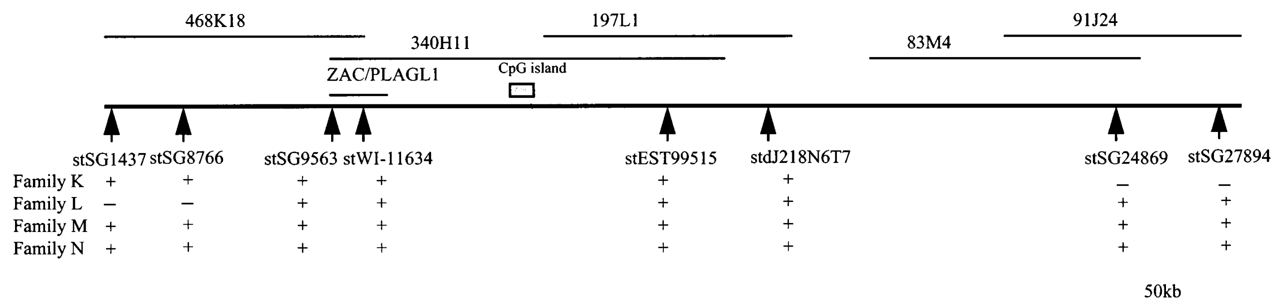

FIG. 1. The TND critical region. The figure shows the overlap of PACs and the positions of the markers (STSs and ESTs) used for quantitative PCR. The results for each family demonstrated to have a submicroscopic duplication are shown. The differentially methylated CpG island is shown along with the gene ZAC/PLAGL1. The figure is based on data from Gardner et al. (21) and was obtained from the Sanger Centre's chromosome 6 mapping and sequencing projects. –, Nonduplication; +, duplication.

boys and girls were approximately equal in the sample group (16 girls, 14 boys), and no consistent pregnancy complications other than intrauterine growth retardation were reported in this cohort. The mean birth weight was 2.1 kg at a mean gestation of 39 weeks. A total of 83% of the patients had a birth weight at or below the 2nd percentile for gestational age. The placenta was recorded as normal in 2 patients and small and of gritty consistency in 3 patients, and histological evidence of a rare placental tumor (a choriohemangioma) was reported in patient 8 (Table 3). A total of 7 patients had macroglossia, and 2 patients had umbilical hernias. These cases did not derive from any particular etiological group.

The median day of presentation was day 3 with a range from a few hours of age to 31 days. Insulin or C-peptide mea-

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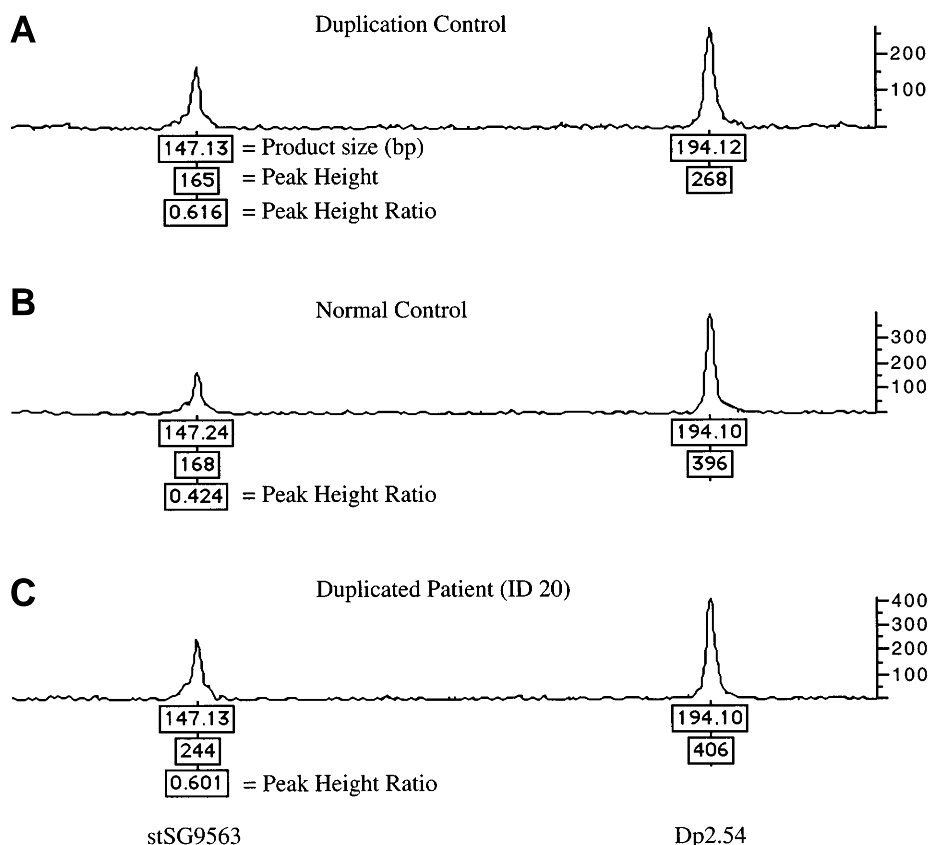

FIG. 2. This is an example of the detection of a submicroscopic duplication using fluorescent quantitative PCR of the EST stSG9563. The 2 peaks seen on the trace represent the products of 2 PCR reactions: the EST stSG9563 from chromosome 6 (147 bp in length) on the left and an internal control PCR from chromosome 5, Dp2.54 (194 bp in length), on the right. In the case of each labeled peak, the top number is the size of the PCR product in base pairs, whereas the other is the peak height measured in fluorescent units against the scale on the right. The ratio of products is calculated by dividing the peak height of stSG9563 by that of the control in the same reaction. When the genomic target sequence of the chromosome 6 marker is duplicated (control trace [*A*] and patient trace [*C*]), its peak height (and therefore the peak height ratio) is higher than that obtained from a control sample (*B*) containing no duplication (~1.5:1).

TABLE 2
Results of an experiment using fluorescent quantitative PCR for 1 critical region marker in the proband of each family with a submicroscopic duplication

Family ID	Identification	Peak height ratio	Interpretation
Family K (marker stSG1437)	Control duplicated	1.47	1.400 ± 0.133 (1.179–1.621)
	Control duplicated	1.27	
	Control duplicated	1.46	
	Control nonduplicated	0.91	0.913 ± 0.025 (0.864–0.962)
	Control nonduplicated	0.94	
	Control nonduplicated	0.89	
	ID 12	1.29	Duplicated
Family L (marker stSG9563)	Control duplicated	1.98	1.955 ± 0.035 (1.886–2.024)
	Control duplicated	1.93	
	Control nonduplicated	0.99	1.067 ± 0.071 (0.928–1.206)
	Control nonduplicated	1.08	
	Control nonduplicated	1.13	
	ID 15	1.96	Duplicated
Family M (marker stSG99515)	Control duplicated	3.40	3.343 ± 0.182 (2.986–3.700)
	Control duplicated	3.49	
	Control duplicated	3.14	
	Control nonduplicated	2.03	2.03 ± 0.180 (1.677–2.383)
	Control nonduplicated	2.21	
	Control nonduplicated	1.85	
	ID 19	3.10	Duplicated
Family N (marker stSG9563)	Control duplicated	0.64	0.613 ± 0.074 (0.468–0.758)
	Control duplicated	0.67	
	Control duplicated	0.53	
	Control nonduplicated	0.45	0.457 ± 0.021 (0.416–0.498)
	Control nonduplicated	0.48	
	Control nonduplicated	0.44	
	ID 20	0.62	Duplicated

Data are means ± SD (95% CIs), unless otherwise indicated. Peak height ratios for duplicated and nonduplicated control subjects are shown with the result obtained for the proband in the same PCR reaction. The SD and 95% CIs were calculated from the 2 control groups. Patient results were reported as duplicated if the peak height ratio fell within the duplication control CI and nonduplicated if the peak height ratio fell within the nonduplicated control CI.

surement was made at the time of diagnosis in 11 cases and was low or negligible in the presence of significant hyperglycemia. Ketones were not present in the urine of all patients tested. Islet cell antibodies were negative in all cases ($n = 13$). The median duration of exogenous insulin requirement was 12 weeks with a range from 4 to 60 weeks (mean of 16 weeks), and age at recovery coincided with a normalization of growth parameters.

Recurrence of diabetes occurred in 11 of 18 patients >4 years of age. The average age at recurrence was 14 years (range 4–25 years). A total of 8 patients have required treatment with insulin, although in 2 cases, this requirement is intermittent (Table 3, ID 25 and 28), and the daily requirements in the remainder are less than those usually necessary for type 1 diabetes. Two patients with treated with diet alone, and 1 was treated with a sulfonylurea. The details of treatment are not known for 1 patient. In 3 young cases, intermittent hyperglycemia has been documented during an intercurrent illness (Table 3, ID 8, 26, and 29), which suggests some disordered pancreatic function. The oldest patient with no evidence of recurrence is 32 years of age. No complications of diabetes are reported in any of the patients. The oldest diabetic patient in this series is 36 years of age and has had diabetes for 16 years.

Growth and development were normal in all but 4 patients, and likely explanations for delays were available for 3 of these patients. One patient (Table 3, ID 2) has low intelligence, which is consistent with a known abnormal karyotype (47,XXX). Patient 8 (Table 3) has a supernumerary chromosome (7) and mild behavioral difficulties noted by 5 years of age. Patient 21 (Table 3) presented at 9 days of age with dehydration and collapse at diagnosis. Patient 27 (Table 3) has spastic tetraplegia and profound retardation with no obvious explanation. Puberty has been reported as normal when recorded in all but the 1 patient with 47,XXX in whom puberty occurred prematurely at 9 years of age. Individuals with duplication of 6q have reproduced successfully, but no children have been born to the UPD cases.

Correlations were determined for several variables in the sample. The only significant correlation was between early age at recovery and late age at recurrence of diabetes ($r = -0.734$, $P = 0.016$, $n = 10$) in the 10 patients for whom the analysis was possible. No other obvious correlations and notably no relationship between birth weight and any other variable were evident. Stepwise regression analysis of the data using stratified birth weight and age at recurrence of diabetes as dependent variables did not reveal any further relationships within the data, possibly because of the small sample size.

Family history. Family history of diabetes is shown in Table 4. No family history of TND was ascertained in any of the 23 sporadic cases. In 1 family (family L) previously

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TABLE 3
Clinical and molecular findings in patients with TND

ID	Family ID	Reference	Sex	Gestation (weeks)	Birth weight (kg)	Percentile birth weight	Dysmorphic findings	Age at presentation (days)	Insulin detected at presentation	Islet cell antibody detected	Age of recovery (weeks)	Recurrence if patient was >4 years	Age at recurrence (years)	Current age (years)	Chromosome 6 abnormality	Karyotype
1	A	—	F	41	2.25	0.4	—	15	—	—	—	Yes	25	29	Pat UPD6	46,XX
2	B	32	F	40	2.50	2	Macroglossia	14	—	—	60	Yes	4	13	Pat UPD6	47,XXX
3	C	33	M	37	1.86	0.4–2	—	6	—	—	20	N/A	N/A	13	Pat UPD6	46,XY
4	C	33	M	37	1.95	0.4–2	—	6	—	—	20	N/A	N/A	13	Pat UPD6	46,XY
5	D	32	M	36	2.00	2–9	Macroglossia and umbilical hernia	31	—	—	5	No	N/A	13	Pat UPD6	Not analyzed
6	E	8	M	40	1.84	<0.4	None	1	Low	—	8	N/A	N/A	4	Pat UPD6	Not analyzed
7	F	11	F	38	1.74	<0.4	None	2	—	Negative	7	N/A	N/A	2	Pat UPD6	46,XX
8	G	7	F	38	1.80	<0.4	Macroglossia and umbilical hernia	1	Low	Negative	10	No	N/A	6	Pat UPD6	46,XX/47,XX,+r(6)
9	H	7	F	40	1.70	<0.4	—	2	Undetectable	Negative	24	Yes	13	Unknown	Pat UPD6	46,XX
10	I	—	M	40	2.01	<0.4	—	3	—	Negative	24	Yes	16	17	Pat UPD6	Not analyzed
11	J	—	F	38	1.94	0.4	—	1	—	—	7	N/A	N/A	1	Pat UPD6	46,XX
12	K	29	M	40	2.15	<0.4	—	3	Undetectable	—	12	Yes	20	Unknown	Dup	Not analyzed
13	K	—	F	37	1.85	<0.4	None	1	Undetectable	—	8	No	N/A	10	Dup pat	Not analyzed
14	K	—	M	42	2.42	9	None	1	—	—	5	N/A	N/A	8	Dup pat	Not analyzed
15	L	30	M	40	1.84	<0.4	None	1	—	—	18	No	N/A	10	Dup pat	46,XY
16	L	31	M	42	2.15	<0.4	None	19	—	—	15	No	N/A	36	Dup pat	46,XY
17	L	31	M	40	2.36	0.4–2	None	3	—	—	14	No	N/A	34	Dup pat	Not analyzed
18	L	31	M	40	2.04	<0.4	None	3	Undetectable	—	18	No	N/A	29	Dup pat	46,XY
19	M	—	F	40	1.60	<0.4	—	7	—	Negative	24	Yes	8	12	Dup pat	46,XX
20	N	4	F	40	1.85	<0.4	—	14	—	—	6	Yes	20	36	Dup	46,XX
21	O	4,15	F	38	2.67	9–25	Microcephaly	9	Low	Negative	12	N/A	N/A	4	Dup pat	46,XX,der(2)ins(2;6)*
22	P	—	F	36	1.66	0.4–2	Macroglossia	1	—	—	7	N/A	N/A	2	Dup pat	46,XXinvDup(6) (q22q23)
23	Q	—	M	42	2.26	<0.4	None	17	Undetectable	Negative	28	N/A	N/A	10	Methylation defect	46,XY
24	R	—	F	—	—	—	—	28	—	Negative	44	Yes	8.5	13	ND	46,XX
25	S	—	F	39	2	<0.4	Macroglossia	8	Undetectable	Negative	12	Yes	9.5	15	ND	Not analyzed
26	T	—	M	38	2.42	2–9	None	5	Low	Negative	12	N/A	N/A	3	ND	46,XY
27	U	—	F	40	2.16	<0.4	Macroglossia	2	—	—	4	Yes	15	28	ND	46,XX
28	V	4	M	40	3.37	50	—	—	—	Negative	4	Yes	17	25	ND	46,XY
29	W	4	F	38	1.96	0.4	—	3	Low	Negative	12	N/A	N/A	3	ND	46,XX
30	X	4	F	38	1.94	0.4	Macroglossia	10	—	Negative	30	N/A	N/A	7	ND	Not analyzed

Patient 12 is the father of patients 13 and 14. Patient 16 is the father of patient 15 and the brother of patients 17 and 18. Dup, duplication of 6q of unknown parental origin; Dup pat, paternally derived duplication of 6q; ND, none dectected; pat UPD6, paternal uniparental isodisomy of chromosome 6. *Now known to include 6q24(21).

TABLE 4
Family history of diabetes

ID	Family ID	Family history of TND	Family history of type 2 diabetes	Family history of type 1 diabetes	Chromosome 6 abnormality
1	A	None	None	None	UPD6
2	B	None	None	None	UPD6
3	C	None	None	None	UPD6
4	C	None	None	None	UPD6
5	D	None	None	None	UPD6
6	E	None	Unknown	Unknown	UPD6
7	F	None	None	None	UPD6
8	G	None	None	None	UPD6
9	H	None	Maternal grandfather	None	UPD6
10	I	None	Maternal grandmother	None	UPD6
11	J	Unknown	Unknown	Unknown	UPD6
12	K	Daughter and son	None	None	Duplication 6
13	K	Father and brother	None	None	Duplication 6
14	K	Father and sister	None	None	Duplication 6
15	L	Father and paternal uncles	Paternal cousin	None	Duplication 6
16	L	Siblings and son	Paternal cousin	None	Duplication 6
17	L	Siblings and nephew	Paternal cousin	None	Duplication 6
18	L	Siblings and nephew	Paternal cousin	None	Duplication 6
19	M	None	Brother	None	Duplication 6
20	N	None	None	None	Duplication 6
21	O	None	None	None	Duplication 6
22	P	Unknown	Unknown	Unknown	Duplication 6
23	Q	None	None	None	Methylation defect
24	R	None	Maternal grandparents	None	None detected
25	S	Unknown	Unknown	Unknown	None detected
26	T	None	Maternal grandfather	Maternal grandmother	None detected
27	U	None	Maternal grandfather	None	None detected
28	V	Unknown	Unknown	Unknown	None detected
29	W	None	Unknown	Unknown	None detected
30	X	None	Father	None	None detected

reported by Temple et al. (16), a paternal cousin of the 3 affected brothers (who had no history of TND) developed gestational diabetes and later diet-controlled type 2 diabetes. She has recently been shown to have inherited a submicroscopic duplication identical to that of her cousins. She inherited this from her asymptomatic father, who is the brother of the probands' father. Patient 19 (Table 4), who had a submicroscopic duplication of 6q24 inherited from her asymptomatic father, has a brother 25 years of age with early-onset type 2 diabetes. He was diagnosed at the age of 14 years, is treated by diet alone, and has also been shown to have a paternal duplication. A family history of type 2 diabetes was identified in 7 patients. In 5 patients, type 2 diabetes was reported in a maternal grandparent. When analyzed by etiological group, 4 of the possible 16 grandparents from the unknown group (no details were available for 3 families) were identified with type 2 diabetes, which is an incidence of 25%. In each case, type 2 diabetes was inherited through the proband's mother. The incidence of type 2 diabetes in the grandparents in the UPD group is 5%, which approximates the quoted national incidence figures of ~2–5% (34).

DISCUSSION

Clinical findings. This study represents the largest cohort of TND patients worldwide and involved an extensive international collaboration (see ACKNOWLEDGMENTS). Most patients presented during the first week of life and had a birth weight below the 2nd percentile. This is in keeping with previous findings (4,12,35). Insulin therapy was required in all but 1 patient (who was treated by rehydration and monitoring of her blood glucose levels with no obvious adverse effects as previously reported) (7). In this cohort, recovery occurred by 15 months of age with all but 2 patients recovering by 9 months of age. That the 2 congenital anomalies reported in this and other studies, macroglossia and umbilical hernia, are also reported in Beckwith-Wiedemann syndrome is intriguing because Beckwith-Wiedemann syndrome is known to result from an imprinting error and is associated with hyperinsulinemia. Christian et al. (10) reported macroglossia to be exclusive to patients with UPD6; however, we found macroglossia to be associated with TND in patients with UPD6, paternal duplications of 6q, and those with no known chromosome 6 abnormality. No trend was clear between birth weight and age at presentation, time of remission, or chance of recurrence of diabetes. The one significant correlation suggests that milder TND may be associated with later age at onset of recurrence of diabetes, and we will be interested to see whether this is confirmed by other studies.

All patients were negative for islet cell antibodies when the test was performed, which supports the theory that the etiopathogenesis is not an autoimmune process as seen in classic childhood type 1 diabetes. None of the patients have undergone surgery or died, and therefore no pathological examination of the pancreas has been undertaken. Further

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evidence indicating that the condition is more akin to type 2 diabetes is the variability of treatment modalities at the time of recurrence of diabetes. The surprisingly low requirement for exogenous insulin, the intermittent nature of the therapy, and the finding of insulin resistance in 2 cases, as discussed by Shield et al. (4), are also in keeping with type 2 diabetes rather than classic type 1 diabetes. The risk of recurrence of diabetes cannot be determined from a study such as this because of a bias toward ascertainment of patients in whom recurrence has occurred.

Subsequent growth, development, and puberty have been normal in all patients except 4, and in 2 of those, a second genetic anomaly (47,XXX and a supernumerary marker chromosome) was discovered. The neurological abnormalities of 1 patient are likely to have resulted from profound dehydration as a result of TND.

Genotype/phenotype comparisons. No apparent phenotypic difference was evident among the patients with UPD6, duplication of 6q24, a methylation defect, and no identifiable anomaly of chromosome 6. This lends further support to the hypothesis that the genetic mechanism of TND is similar in most patients (i.e., 2 copies of a paternal allele at 6q24). Other mechanisms causing overexpression of the paternal gene or inappropriate expression of the maternal allele could account for the remaining cases. The additional presence of a maternal allele in the paternal duplication cases appears to have little clinical effect, and, interestingly, no evidence exists that general intellectual development is lower in the duplicated group.

Genetic implications. Genetic counseling should be offered to TND patients. Those patients with paternal UPD would be predicted to have a low sibling and offspring risk. All inherited cases in this series have a paternally inherited submicroscopic duplication, which explains familial recurrence and positive linkage to 6q23–q24, as previously reported (16). Women with the duplication may pass it on, and TND may occur in subsequent generations. Those men with a duplication will have a 50% risk of passing on the duplication and having a child affected by TND. However, these symptoms are not inevitable at birth. This observation follows the paternally transmitted duplication demonstrated in the paternal cousin of the 3 affected siblings (ID 16–18) and the older brother of patient 19, who had no history of TND but developed diabetes in later life. Both cases suggest that the clinical spectrum associated with submicroscopic 6q duplications may be broader than that in typical TND cases and lends support to the possibility that molecular rearrangements or imprinting anomalies at 6q24 may have implications for more common types of diabetes. In the unknown etiologic group and the methylation defect case, the recurrence risk cannot be predicted. The incidence of type 2 diabetes in the grandparents of the probands in the unknown group is surprisingly high and may indicate a shared susceptibility to diabetes in adult life. Of the 5 patients with a maternal grandparent with type 2 diabetes, 3 are from the unknown etiological group. We theorize that mutation of a maternally inherited imprinting control center within 6q24 could cause inappropriate expression of a maternal allele; a precedent for such a disease mechanism exists in Beckwith-Wiedemann syndrome mutations on chromosome 11p (36).

This study identified the cause of TND in 70% of sporadic cases and 100% of familial cases and highlighted the clinical findings in a large cohort of patients. We predict that identification of the gene for TND will yield important insights into early pancreatic development. Low or undetectable insulin levels in TND at presentation with subsequent recovery suggest that the gene product delays the production or release of insulin. Speculating on its role in the low birth weight of TND patients and subsequent development of adult diabetes is tempting. The genes responsible for maturity-onset diabetes of the young have been shown by Hattersley et al. (37) to have a similar effect, and the gene for TND may be a further example of a gene with a function in fetal life that plays a role in adult-onset disease.

ACKNOWLEDGMENTS

R.J.G. are D.J.G.M. are supported by the British Diabetic Association.

We are grateful to the following people for sending samples and patient details from the patients discussed in this study: Prof. C. Polychronakos, Dr. M. Gonthier, Dr. G. Valerio, Dr. A. Franzese, Dr. M. Salerno, Dr. F. Neilsen, Dr. M. Whiteford, Dr. S. Hyers, Dr. L. Lamont, Prof. J.D. Baum, Dr. A. Ferguson, Dr. M. Kibirige, Dr. J. Zlotogora, Dr. J. Challener, Dr. P. Swift, Dr. M. Barrow, Dr. J. Clayton-Smith, Dr. S. Price, Dr. D.J. Carson, Dr. G. Hendrickx, Prof. J.-P. Fryns, Dr. U. Schimmel, Dr. G. Hawthorne, Dr. J. Storr, Dr. P. Betts, Dr. A. Piesowicz, Dr. R. Ironton, Dr. P. McNally, Dr. K.J. Robertson, Dr. P.D. MacDonald, Dr. D. Abeliovich, and Dr. A. Gorsuch. Other collaborators to the TND project include Dr. S. Zneimer, Dr. B. Ziel, Dr. K.E. von Muhlendahl, Dr. R. Hennekam, Dr. D.I. Johnston, Dr. C.S. Smith, Dr. P.W. Fowlie, Dr. K.M. Adler, Prof. V. Fellman, Dr. A. Medeira, Dr. C. Garrett, Dr. M. Parslow, Prof. M.J. Macdonald, Dr. J. Goodship, Dr. S. Kant, Dr. D. Morin, Dr. Z. Ahmed, Dr. J. Wales, Dr. H. van Hooff, Prof. D. Gill, Dr. S. Holder, Dr. Shirsalkir, Dr. D.J. Beale, and Dr. N. van Regemorter. Colleagues in the Wessex Genetics Service who have helped with this study include Dr. N.R. Dennis, Dr. J.A. Crolla, Dr. R.S. James, and H. Bullman. We also thank A.J. Mungall and Dr. I. Dunham at the Sanger Centre, Dr. A. Collins for the statistical analysis, and Prof. P.A. Jacobs and Prof. I.N.M. Day for their advice and support.

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