



SHORT REPORT

Variable features of transient neonatal diabetes mellitus with paternal isodisomy of chromosome 6

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We describe two patients who suffered transient neonatal diabetes mellitus (TDNM), due to paternal isodisomy of chromosome 6. One patient, now 5 years old, had severe intra-uterine growth retardation, but recovered normal growth parameters. The other patient, currently 12 years old, had a normal birth weight but showed impaired post-natal growth; in addition to TDNM the patient presented with cardiac and thyroid abnormalities. These cases may suggest that the clinical phenotype of TDNM is more variable than previously believed. The contribution of genetic and epigenetic factors needs to be determined to elucidate the phenotype–genotype relationships of this disease. *European Journal of Human Genetics* (2000) 8, 137–140.

Keywords: neonatal diabetes mellitus; chromosome 6 uniparental disomy; imprinting; intra-uterine growth; post-natal growth; developmental abnormalities

Introduction

Neonatal diabetes mellitus (NDM) is a very rare entity with an estimated incidence of 1/400 000–1/500 000 births in Europe.¹ Neonates with NDM have an absolute requirement for insulin to maintain blood glucose. In about half of the NDM cases, the diabetes is transient (TNDM, MIM 601410). TNDM is frequently associated with intra-uterine growth retardation (IUGR) and very low adiposity. TNDM usually resolves before 6 months, but type-2 diabetes mellitus has been reported later in life. Chromosome 6 duplications,^{2,3} and paternal uniparental disomy of chromosome 6 (UPD6), isodisomy, have been described in patients with TNDM.^{4,5} A candidate region on 6q23–q24 has been identified with an imprinted gene thought to be involved in the maturation of pancreatic beta-cells.⁶ We report two further cases of TNDM with paternal UPD6, with different growth patterns and clinical abnormalities.

Patients and methods

Clinical report

Two children were diagnosed with idiopathic TNDM. Table 1 shows the main clinical features and laboratory findings.

Child A is the first child of unrelated parents of Moroccan origin, the younger sister is unaffected. The plasma glucose concentration was 5 mmol/l, 5 hours after birth. Plasma insulin concentration was < 3 mU/l and plasma C peptide concentration 0.16 pmol/l. Insulin was given intravenously at 0.15 mU/kg/day. After cessation of insulin, capillary blood glucose varied between 7 and 12 mmol/l. Follow-up included glycated haemoglobin assays; oral glucose tolerance test and titrations of islet-cell antibodies (ICA, IAA, IA2, GAD) at 1, 2, and 4 years of age were in the normal range. Height and weight reached the main reference values curves by 1 year of age and have remained around this level since.

Child B is the fourth child of consanguineous parents of Moroccan origin. Three older siblings were unaffected. The newborn presented with vital distress requiring external cardiac stimulation. On the first day of life the child presented with hypotonia and seizures which were controlled with phenytoine. TNDM in patient B was associated with other severe pathological features (Table 1). Statural growth is abnormal, height being 134 cm (–2σ) and weight 25 kg (–2.5σ) at age 10 years.

Genotyping

10–20 ml of peripheral blood were drawn from each patient and parents after they gave their informed consent. Genomic DNA was extracted from blood with the Nucleon BACC2 kit

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(Amersham Pharmacia Biotech SA, Orsay, France). For genotyping, microsatellite DNA markers⁷ were amplified. Genotypes were determined with either ³²P-labelled primers or fluorescent primers analysed on an ABI 377 (Perkin Elmer, Applied Biosystems, Foster City, USA) automated sequencer with GeneScan and Genotyper softwares. Each PCR was carried out in a 25 µl reaction mixture containing 20 ng of genomic DNA, 20 pmol of each primer, 1–2 mM MgCl₂, Taq polymerase buffer, 200 mM of each dNTP, and 0.2 unit of Taq polymerase (Gibco-BRL Life Technologies, Cergy Pontoise, France). Samples were amplified in 30 cycles of 20 s at 94°C for denaturing, 20 s at optimum annealing temperature, and 20 s at 72°C for extension, in a GeneAmp 9600 thermocycler (Perkin-Elmer). Alleles were separated by electrophoresis on a 5% denaturing polyacrylamide gel for 2.5 hours in a model ABI 377 DNA sequencer (Perkin-Elmer). For some markers, the genotype was determined by labeling one of the PCR primers with α-³²P-dATP by T4 polynucleotide kinase. PCR conditions were the same as those used for fluorescent-labelled markers.

Results

The results of genotyping with 21 microsatellite markers distributed along the whole of chromosome 6 are given in Figure 1. The marker alleles were apparently homozygous all along chromosome 6 in both patients. The inherited alleles were of paternal origin with no maternal contribution – evidence of a paternal UPD6. The paternal isodisomy is demonstrable at 10 loci for child A and 14 loci for child B (Figure 1). Markers which were not fully informative were

inherited in a pattern consistent with homozygosity for the paternal allele. Markers from other chromosomes including 20 markers on chromosome 11 (not shown), were inherited in a Mendelian mode. Routine karyotypes were normal.

Discussion

We report two new TNDM cases with paternal isodisomy of chromosome 6. UPD6 has previously been reported to be associated with various clinical features such as complement deficiency,⁸ NDM with methyl malonic acidemia and pancreatic β cells agenesis,⁹ and TNDM with macroglossia.¹⁰ The association between TNDM and paternal UPD6 has been observed in at least six independent cases in various ethnic groups.^{4,5} These recent reports and our observation are in favour of the involvement of imprinted gene(s) on the chromosome region 6q23–q24.⁶ Chromosome 6 paternal isodisomy is found in approximately 20–30% of patients with TNDM. It is thus a common genetic mechanism of the disease, but different from that involved in the Beckwith-Wiedemann and Prader-Willi/Angelman syndromes in which the UPD described are segmentary and located at the 11p15.5 and 15q11–q13 imprinted domains, respectively. Paternal duplications of the 6q23–qter chromosomal region found in some patients with TNDM,^{2,3} suggest that a gain of function of a paternally expressed gene may be involved. Maternal UPD6 has been reported in two cases with no evidence of TNDM:¹¹ once in a genome-screen linkage study, and once in HLA typing.^{11,12} The apparently low incidence of TNDM in the European population could be due to patients not requiring insulin therapy at birth going undiagnosed.¹³

Table 1 Clinical features and laboratory findings of patients with TNDM

Clinical features	Patient A	Patient B ^a
Sex	female	female
Age (yr)	5	12
Birth weight (g)	1670	2800
(centile)	<3rd	10th
Birth length (cm)	41.5	50
Birth head circumference (cm)	31	35
Gestation (weeks)	41	41
Intra-uterine growth	retarded	mild retardation
Placenta	calcified	oligoamnios
Apgar score	10 at 1 min	3 at 1 min, 10 at 10 min
Infusion feeding	birth to 72 h	none
Hyperglycaemia, dehydration	day 4: 9.5 mmol/l day 10: 14–22 mmol/l	48 h: 19 mmol/l
Insulin interrupted at	22 days	2½ months
Intellectual development	normal	school delay
Postnatal growth	normal	slowed
Diabetes relapse	no	no
Thyroid	–	hypothyroidism ^b
Cardiac	–	mitral insufficiency ^c
Other	–	β-thalassaemia major ^d
Diabetes in the family	none	IDDM, NIDDM ^e

^aThe report on patient B of this paper is a reappraisal of a case previously reported as fortuitous UPD6 by Bittencourt MC *et al*¹²; ^bdiagnosed at 11 years, TSH 326 mmol/L, free T4 0.4 pmol/L (puffed-up face, cold intolerance, astheny), treated with L-thyroxine; ^cMitral valvular insufficiency, with left ventricular dilatation, diagnosed at 8 years, treated with captopril; ^dBeta-thalassaemia diagnosed at 4½ years, required blood transfusions, deferoxamine infusion, a splenectomy at 10 years; ^eMaternal grandmother with IDDM, maternal grandfather with DNID.

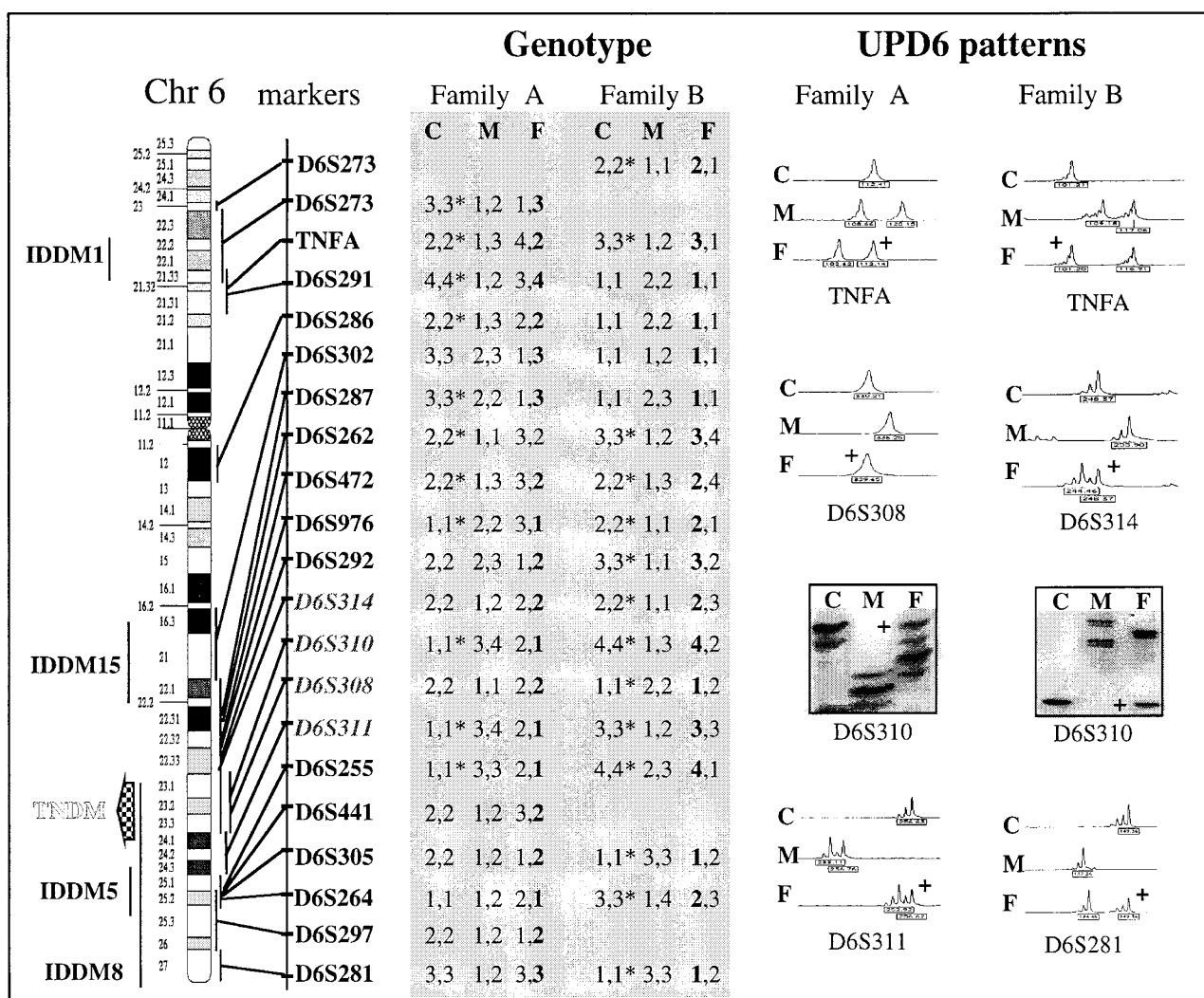


Figure 1 Genotype for 21 chromosome 6 microsatellite markers of patients with TNDM. Paternal haplotype transmitted in bold. Right part: alleles of representative markers in families A and B, Genotyper sample analysis for all the markers except D6S310 (autoradiography). Patient A (TNF α , D6S308, D6S310, D6S311), patient B (TNF α , D6S314, D6S310, D6S281). The affected daughter in both families has inherited only paternal alleles. The inherited paternal allele is indicated by +. Symbols: (C): affected child; (M): mother; (F): father. Left part: chromosome 6 ideogram, cytogenetic location of markers and of loci predisposing to insulin dependent diabetes mellitus (IDDM, type 1 diabetes), IDDM1, IDDM5, IDDM8, IDDM15, as well as the candidate region of the TNDM locus (markers in italic).

IUGR and low birth weight in TNDM could result from the lack of insulin as a growth factor *in utero*. It would be valuable to identify genes participating in birth weight control, since low birth weight is a condition that reduces perinatal survival and increases the risk of cardiovascular disease, and type 2 diabetes in adult life.¹⁴

TNDM in patient A is typical of 'classic TNDM',¹⁵ after 5 years she is healthy. Patient B had impaired statural and ponderal post-natal growth and other pathological features (cardiac anomalies and hypothyroidism) in addition to TNDM. Although hypothyroidism may be due to complications of major β thalassaemia,¹⁶ there are alternative possible

explanations: unmasking of an autosomal recessive mutation due to the chromosome 6 isodisomy, or consanguinity of the parents resulting in a recessive disorder. Other mechanisms such as i) a multiendocrine alteration process, ii) imbalance in signalling, iii) imprinting effects (target tissue, variable tissue responses to stimuli, the mutation type, and the parental origin of the allele which bears the mutation), are also possible.

Further investigations are needed to identify the clinical spectrum of TNDM, its genetic and molecular basis, and to evaluate its impact on more common forms of diabetes such as non insulin-dependent diabetes mellitus (NIDDM).

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