Further evidence for an imprinted gene for neonatal diabetes localised to chromosome 6q22–q23

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Received March 6, 1996; Revised and Accepted May 7, 1996

Transient neonatal diabetes mellitus (TNDM) is a rare form of childhood diabetes which usually resolves in the first 6 months of life but which predisposes to type 2 diabetes of adult onset. We recently reported paternal uniparental isodisomy of chromosome 6 (UPD6) in two children with TNDM and proposed that there may be an imprinted gene important in the aetiology of diabetes on chromosome 6. We now describe two unrelated families which independently suggest that the gene is imprinted, is paternally expressed and maps to 6q22–q23. One family has a duplication while the other, with familial TNDM, shows linkage to a marker in this region.

INTRODUCTION

Transient neonatal diabetes mellitus (TNDM) occurs in approximately 1/500 000 births (1). Patients are usually born with a degree of intra-uterine growth retardation and present within the first six weeks of life with severe failure to thrive, hyperglycaemia and dehydration. Exogenous insulin therapy is usually required to maintain euglycaemia until the condition resolves at a median age of 4 months. There is evidence of failure of normal insulin secretion in response to glucose loading (2). This condition can predispose to adult onset diabetes, with numerous cases reported in the literature (1). There is evidence that the aetiopathogenesis of the later onset diabetes is not the classical autoimmune related type 1 form of childhood but is consistent with type II diabetes with insulin resistance (3). There has been no unifying hypothesis to explain this condition, but we recently reported two TNDM patients with paternal uniparental isodisomy of chromosome 6 and hypothesised that there was an imprinted gene on chromosome 6 involved in normal pancreatic development (4).

Subsequently we became aware of two children with multiple congenital anomalies and neonatal diabetes with a partial duplication of the long arm of chromosome 6. The area of overlap of duplication in these cases was 6q23 (5,6). The parental origin was determined in one case and was shown to be paternal (5). This observation, in conjunction with the evidence of TNDM occurring in paternal UPD6, implies that paternal duplication rather than maternal deletion of a region of chromosome 6 results in TNDM.

During a systematic search for abnormalities of chromosome 6 in 10 British cases of TNDM, we discovered one patient with classical TNDM and a paternal duplication of a small region of chromosome 6q, the inheritance pattern of which was consistent with the role of imprinting in this condition (family A). Subsequently we studied a second family with four and possibly five affected individuals (family B) and showed linkage of the condition to a marker at 6q23.

RESULTS

Family A: an inherited duplication of 6q in three generations

The proband was the first child (female) of unrelated Caucasian parents. She was born at 38 weeks gestation weighing 2.67 kg (3–10th centile) and was admitted to hospital at 9 days of age. Her weight had fallen to 2.1 kg (<0.4th centile) and she had profound dehydration and a raised blood sugar of 74 mmol/l (normal range 2.8–5.0). The child required intravenous insulin therapy (0.4 units/kg/day) to maintain euglycaemia. Ultrasound of the pancreas was normal but C-peptide levels measured at presentation showed markedly inadequate insulin production at 0.06 nmol/l (normal range 0.1–0.6) in the face of severe hyperglycaemia. Anti-islet cell antibodies were absent. The child had microcephaly with a head circumference below the third centile and myoclonic seizures developed soon after admission which were controlled successfully with anti-convulsants. Insulin therapy was required for a total of 3 months. The child at 7 months of age currently remains euglycaemic without insulin therapy. A recent HbA1c measurement was within the normal range at 5.6% (normal range 4.5–6.7%). Her weight is currently on the 10th centile. She is microcephalic but not otherwise dysmorphic and is developmentally delayed.

PCR analysis of dinucleotide repeat polymorphisms located on 6q detected a duplication in the proband, her father and paternal grandmother. Evidence of the duplication was provided either by the presence of three alleles, or increased dosage of one allele, at

**Figure 1.** Analysis of family A using polymorphic dinucleotide repeat polymorphisms. (a) D6S292 and (b) D6S403. In each case the pedigree and genotype are given above the autoradiogram. At D6S292, three alleles are present in the proband and paternal grandmother and an increased dose of the larger allele is present in the father; with this technique the smaller alleles are preferentially amplified, as can be seen in the proband and her heterozygous mother (II.1). In the proband’s father (II.2) the equal intensity of the two alleles therefore suggests duplication of the larger allele. At D6S403 three alleles are present in the proband, her father and her paternal grandmother.

Cytogenetic analysis revealed apparently normal chromosomes 6 but FISH analysis using a whole chromosome paint for chromosome 6 demonstrated a subtle insertion of additional material from chromosome 6 into the short arm of chromosome 2. The proband’s karyotype was 46,XX, der(2) ins(2;6)(2pter-2p22.2::6q22.32-q23.1::2p22.2–2pter) pat. ish der(2) ins(2;6)(wcp6+) (Fig. 2).

The proband’s father and paternal grandmother, both carrying the insertion duplication, are healthy adults, with normal head circumference and growth and with no history of diabetes or unexplained neonatal illness. The father’s birth weight was 4.3 kg and he has a normal oral glucose tolerance test (OGTT) and C-peptide level. He has mild learning difficulties but the paternal grandmother is of normal intelligence. The duplication in the proband’s father and grandmother appeared identical to that in the proband using both cytogenetic and molecular techniques.

Family B: dominant inheritance of TNDM

Family B has been previously reported by Ferguson and Milner (9), Milner et al. (2) and Wilson (10) (Fig. 3). Brothers II.2, II.3 and II.4 are now aged 32, 30 and 25 years of age, respectively, and are healthy with no symptoms of diabetes. III.1 also developed classical TNDM. He is now well with no symptoms of diabetes aged 6 years and he has an unaffected younger sister.

Individual II.6 was born at term with a birth weight of 2.3 kg. She was described by her parents as being a sick infant with poor weight gain and recurrent illness. She was born before the birth of her affected cousins and TNDM was not suspected. The history is compatible with undiagnosed TNDM as occasional patients have been reported with TNDM who do not require exogenous insulin therapy (11). At 28 years of age she developed gestational diabetes which resolved after the pregnancy. Mild diabetes which continues to be controlled by diet alone developed 4 years later and is monitored annually. Her blood sugars are mildly raised (maximum 7.8 mmol/l; normal range is 2.8–5.0 mmol/l) and her maximum HbA1c was 8.3% (normal level is 5.6% ± 1.1). This mild adult onset diabetes is typical of TNDM patients in later life.

The karyotypes of the parents and affected individuals of the family, using extended G banding and fluorescence in situ hybridisation (FISH) with chromosome 6 paint, were normal. Furthermore no duplication, deletion or abnormal inheritance of chromosome 6 could be seen using molecular techniques. However, positive linkage with D6S310 assigned to 6q23.3 was demonstrated (Fig. 3). A positive lod score of 1.5 at θ = 0 is generated if calculated including only the members with classical TNDM. It seems reasonable however to add the genetic data from the paternal cousin with adult onset diabetes and possible TNDM, and this increases the lod score to 2.7 at θ = 0. This figure is significant as the locus has already been implicated in this condition in family A. Analysis with other markers found to be duplicated in family A showed one recombination with D6S292 and a lod score of 1.1 at θ = 0.125, and D6S403 showed no recombination and a lod score of 1.8 at θ = 0.

**DISCUSSION**

We have previously suggested that TNDM may be due to an imprinted gene on chromosome 6 (4). This followed the discovery of paternal uniparental isodisomy of chromosome 6 in three patients, two with TNDM (4), and a third with neonatal diabetes who died soon after birth (12).

Family A, in which the affected individual had classical TNDM and an unbalanced paternal duplication of chromosome 6, defines the area of interest as that encompassed by the duplicated markers D6S292, D6S403, D6S310, D6S308, (6q22.33–6q23.3) bracketed by markers D6S472 at 6q22.31 and D6S311 at 6q24.1 which were not duplicated. Positive linkage in family B with...
In family A apparently identical duplications of the critical region of chromosome 6 have been inherited by three individuals, only one of whom had TNDM. It is unlikely that the diagnosis of TNDM has been missed in the father and grandmother of the proband in view of their normal neonatal histories. The proband’s father shows no evidence of subclinical diabetes or insulin resistance at age 21 years reflected in a normal OGTT and C-peptide level. It is possible that the duplication is unrelated to the clinical features although the accumulating evidence implicating this region of chromosome 6 makes this extremely unlikely.

The pattern of inheritance of TNDM in family A can be explained by the presence of an imprinted gene. If the gene is expressed exclusively from the paternal homologue both paternal UPD6 and paternal duplications of the critical region of chromosome 6 would result in over-expression of the gene and an identical clinical picture. The affected proband with TNDM inherited two copies of the critical region from her father, and thus has over-expression of the imprinted gene, whereas the father who inherited an apparently identical duplication from his mother had an extra copy of a ‘silent’ gene with no phenotypic effect. The inheritance of the duplication in the grandmother is unknown but we would predict it to be maternal in origin. We cannot explain why the proband in family A has, in addition to TNDM, microcephaly and mental retardation, the father has mild developmental delay and the grandmother is of normal intelligence, despite all three individuals having the same unbalanced karyotype. There may be a cluster of imprinted genes in this region as seen at other imprinted regions in the human genome to explain the proband’s retardation, but then we would expect a similar phenotype in the other TNDM cases with paternal UPD6. One possible explanation is that the region of duplication contains few expressed genes and the retardation in the proband is a result of her profound neonatal hyperglycaemia. It is difficult to explain why the phenotype of the father differs from that of the grandmother, but it is feasible that his retardation is due to an alternative cause.

Our observation is compatible with other cases involving 6q duplications. There have been three reported cases with proven paternal inheritance. The child described by Pivnic et al. (5) had a duplication involving 6q23-qter, died at 2 months of age but had neonatal diabetes and multiple congenital anomalies. A second male infant with a similar duplication died at birth. There are no comments on the pancreatic histology but the child had severe intra-uterine retardation which is compatible with TNDM (13). The female infant described by Chase et al. (14) had multiple congenital anomalies and no evidence of diabetes but the duplicated region (6q24-qter) was outside our region of interest. There has been one other case to our knowledge with a duplication of 6q involving 6q21–23 with TNDM but the parent of origin is unknown (6).
Figure 3. Analysis of family B with polymorphic marker D6S310. The pedigree and genotypes of all individuals are given above the autoradiogram. All individuals affected with transient neonatal diabetes have inherited allele 6 from their father. The unaffected individuals, with the exception of individual II.6, have inherited a different paternal allele. II.6 has type 2 diabetes and has inherited allele 6 from her father. In this family, allele 6 of D6S310 is segregating with the disease.

In keeping with our hypothesis of an imprinting mechanism, neonatal diabetes has not been reported in association with 18 maternal duplications of 6q in the literature (5). In reports of chromosome 6 deletions in this area, reviewed recently by Pandya et al. (15), no child is described with neonatal diabetes, which might be expected if the absence of the maternal allele is implicated.

The inheritance seen in family B is also compatible with an imprinting mechanism, as the linked allele is paternal in origin in all affected members of the family, although the result of maternal transmission is unknown. It is assumed that the unaffected grandfather inherited the mutated allele/cryptic duplication from his mother. There is at least one other familial case in the literature which supports the presence of a paternally expressed dominant gene and TNDM. The family reported by Coffey et al. (16) describes three affected sibs born to an unaffected father by three different partners.

Long term follow-up of the patients with TNDM described in this paper has not been possible. However, of the two patients we previously described with paternal UPD6 and TNDM, one developed type 2 diabetes with insulin resistance aged 13 years (3) while the other, currently aged 3 years, although euglycaemic, has evidence of insulin resistance reflected by a raised C-peptide level (data not shown). Linkage studies in family B also suggest that this potential diabetes gene may have a role in the adult onset diabetes seen in the individual II.6 who did not have overt TNDM.

In looking for a possible candidate gene where increased copy number or upregulation of a gene at 6q22–q23 could result in TNDM, the work of Maddux et al. (17) is of great interest. They describe nine patients with type 2 diabetes and insulin resistance, seven of whom have over-expression of a membrane glycoprotein PC1 in skin fibroblasts. As PC1 has been mapped to 6q22–23 (18) and is implicated in diabetes, it should be considered a potential candidate gene.

In conclusion, although TNDM is a rare disorder, the gene for this condition may prove of critical importance in the aetiology of more common types of adult diabetes. We have shown that the causative gene for TNDM lies within the region 6q22.33–q23.3 and is likely to be imprinted.

MATERIALS AND METHODS

Molecular

DNA was extracted from whole blood by a salt precipitation method (19). Primers have been made using phosphoramidite chemistry either on an ABI 392 oligonucleotide synthesiser or by Oswel DNA services. All primer sequences were obtained from Genethon or GDB (7).

One of the PCR primers was end-labelled using T4 polynucleotide kinase prior to PCR amplification.

DNA amplification was carried out in an ABI 9600 multowell programmable thermal cycler by the method of Hudson et al. (20).

All products were separated by denaturing polyacrylamide gel electrophoresis and visualised by autoradiography.

Cytogenetic

Chromosomes were prepared by standard techniques after semi-synchronisation with FdU and release with thymidine (21).

A modification of the method of Pinkel et al. (22) was used for chromosome painting with a whole chromosome paint for chromosome 6 (Cambio). Chromosomes were counterstained with DAPI and viewed through a Zeiss Axiophot microscope. Images were captured with a Photometrics cooled CCD camera and enhanced with Digital Scientific Smart Capture software. A
minimum of five cells were captured in each case and findings corroborated by an independent observer.

ACKNOWLEDGEMENTS

This work was supported by a grant from the British Diabetic Association (R.J.G) and the Wellcome Trust (R.S.J.). We are grateful to Ms Wilson for recontacting family B. Islet cell antibody tests were performed in the laboratory of Professor F. Bottazzo, London. We thank Professor Patricia Jacobs for her invaluable support with this project and Professor Newton Morton and Dr Andy Collins for their help with the chromosome 6 map and calculation of lod scores.

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