**LETTER TO JMG**

**Major difference in aetiology and phenotypic abnormalities between transient and permanent neonatal diabetes**

E Marquis, J J Robert, C Bouvattier, C Bellanné-Chantelot, C Junien, C Diatloff-Zito


Neonatal diabetes (ND) is a rare entity with an estimated incidence of 1/400 000 births in Europe. Hyperglycaemia usually occurs in the first few days of life and patients require insulin treatment. Intrauterine growth retardation, low birth weight, and decreased adipose tissue are frequently associated. ND is permanent in some patients (permanent ND), and in other cases hyperglycaemia is transient (transient ND, OMIM 601410). Type 2 diabetes (T2D) frequently arises in adolescence or adulthood in transient ND patients. Chromosome 6 abnormalities are specifically associated with transient ND, with imprinting effects unmasked by uniparental disomy (UPD) of paternal chromosome 6 and duplications in 6q24. Two imprinted genes expressed from the paternal allele in various tissues, HYMAI (hydridiform mole associated and imprinted transcript), lie in the transient ND locus in 6q24.

The genetic causes of permanent ND forms are less known. Homozygous mutation in the glucokinase gene (GK) and in the insulin promoter factor-1 (IPF1) gene may lead to permanent ND owing to complete deficiency of the GK or IPF1 gene product. Mutations of the eukaryotic translation initiation factor-2-alpha kinase 3 (EIF2AK3) gene were found to segregate with the Wolcott-Rallison syndrome (OMIM 226980), a rare autosomal recessive disorder with early onset permanent diabetes mellitus and multiple epiphyseal dysplasia (spondyloepiphyseal dysplasia). Clinical information and a molecular genetic study of 14 patients with transient or permanent ND forms are reported. The phenotypes of ND patients from previous reports together with cases reported here provide an initial outline for further studies and molecular mechanisms.

**PATIENTS**

Fourteen patients with ND were studied. Their main clinical features are summarised in table 1. The patients were all born at term, mean birth weight was 2288 g (SD 570 g), and nine patients displayed intrauterine growth retardation (>2 SD). Diabetes was diagnosed within the first month of life in 13 of the patients. The proband referred to as O7 is a child from a multiplex family, with permanent diabetes appearing before 6 months of age. Four other members of family O in two generations were affected in a manner suggestive of an autosomal dominant pattern of inheritance. Patients 13 and 14 were brother and sister with permanent ND. All nine patients with transient ND were sporadic cases. All the patients were found to be negative for autoantibodies against islet cells (ICA) or insulin (AIA). Sequences of HLA-DRB1 and DQB1 loci were achieved for all the patients and relatives and alleles classically associated with juvenile diabetes were not found. Ultrasound scans were performed to exclude pancreatic agenesis in permanent ND patients. The patients were all white, except for patient J who originated from Guyana.

**METHODS**

**Molecular genetic studies**

Peripheral blood (10-20 ml) was drawn from each participant. Written informed consent was obtained from all the parents. DNA was prepared and microsatellite marker analysis performed as previously described. Microsatellite markers

---

**Table 1** Main clinical features of patients with insulin dependent neonatal diabetes (ND)

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td>A</td>
</tr>
<tr>
<td><strong>Birth weight (g)</strong></td>
<td>1670</td>
</tr>
<tr>
<td><strong>Age at diagnosis</strong></td>
<td>13 d</td>
</tr>
<tr>
<td><strong>Diabetes form</strong></td>
<td>Tr</td>
</tr>
<tr>
<td><strong>Insulin†</strong></td>
<td>12 d</td>
</tr>
<tr>
<td><strong>Family diabetes history</strong></td>
<td>–</td>
</tr>
<tr>
<td><strong>Congenital birth anomalies‡</strong></td>
<td>–</td>
</tr>
</tbody>
</table>

† = transient ND; ‡ = permanent ND; na = not available.

*Patient M developed non-insulin dependent diabetes at 18 years of age with hyperglycaemia controlled by oral sulphonylurea.

†Duration of insulin therapy in transient ND; d = days, mth = months; T1D = type 1 diabetes in the paternal branch for patients D, I3, and I4 and in the maternal grandparents for patients C and K; T2D = type 2 diabetes in the maternal grandparents for the transient ND patients.

‡Congenital birth abnormalities degree of severity: (−) = absent, (+) = moderate mild, (+++) = marked with a combination of more than two organs affected, details in table 2 [2].
with a heterozygote frequency of about 70% were selected from the Centre d’Etude du Polymorphisme (CEPH-Fondation Jean Dausset, http://www.ceph.fr/ceph-genethon). The genetic location and order of the markers were deduced from the integrated map of the Whitehead Institute (http://www-genome.wi.mit.edu/) and from the integrated cytogenetic and physical maps of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/genemap/). For genotyping, DNA microsatellite markers were amplified by PCR in a GeneAmp 9600 thermocycler (Perkin-Elmer). The PCR products were then analysed with GeneScan and GENOTYPER software (Applied Biosystems-(ABI)-Perkin Elmer, USA) after separation of the alleles by electrophoresis on a 6% denaturing polyacrylamide gel for two and a half hours in a model ABI 377 DNA sequencer (ABI-Perkin-Elmer). The markers for chromosome 6 are listed in fig 1B.

The exons encoding the GK gene were screened for mutations in members of family O and in patients B, C, K, M, and N with T2D in relatives by direct sequencing. The insulin promoter factor-1 (IPF1) exons were analysed by direct sequencing of polymerase chain reaction (PCR) products in all the patients.

RESULTS
Molecular studies
We found no mutation in the GK or IPF1 genes in the genomic DNA of the patients examined. The genotypes of the patients and their parents were determined using 24 polymorphic microsatellite markers covering the entire length of chromosome 6. Paternal isodisomy of chromosome 6 was found in two transient ND (22.2%) patients. One paternal allele and the complete absence of a maternal allele were found in cases A and C (fig 1A). All of the chromosome 6 markers were homozygous along the length of chromosome 6 (fig 1B). The other patients with transient or permanent ND displayed normal biparental inheritance of alleles (not shown). Additional markers tested for patients F, G, I3, I4, K, and N with homozygosity for either D6S308 or D6S1703 ruled out the presence of

Figure 1  [A] Paternal chromosome 6 isodisomy in two transient ND patients, A and C. Chromosome 6 microsatellite markers D6S409 (case A) and D6S1703 (case C). C=affected child, Mo=mother, Fa=father. The patients have inherited one paternal allele and no allele from the mother. This was observed for all the chromosome 6 markers examined, covering the entire length of the chromosome. (B) Screening for a common region of homozygosity for chromosome 6 microsatellite markers in both transient ND (underlined) and permanent ND patients. The presence of contiguous homozygous markers could be used to identify a disomic region. The genotyping results are shown in terms of allele homozygosity. Black circle=homozygosity, black square=heterozygosity. The marker position in centimorgans (cM) is indicated on the left. Non-informative markers are shown by empty symbols.

Letters

www.jmedgenet.com
for marker D6S409, point linkage analysis gave a maximum lod score of multipoint calculation in pedigree I. In pedigree O, lod scores a partial paternal uniparental disomy in the transient ND component of the LINKAGE package version 5.1. Multipoint linkage analyses were performed using the MLINK shown in fig 2. In each family, the affected patients inherited D6 and between D6S286 and D6S287 on the paternal chromosome I4 between D6S255 and D6S305 on the maternal chromosome guineous; a meiotic recombination occurred in patients I3 and ing chromosome 6 microsatellite markers. Family I was consan-

Figure 2  Results of dinucleotide repeat marker analysis for chromosome 6 in two familial permanent ND cases, families I and O. Markers are ordered from pter to qter (top to bottom) and are shown on the left. The haplotypes are shown with one possible phasing. Filled symbols: patients with permanent ND. Deduced haplotypes for subject 11 are shown in brackets.

Table 2  Phenotypes and developmental abnormalities of patients with neonatal diabetes (ND)

<table>
<thead>
<tr>
<th>Patients</th>
<th>Phenotypes</th>
</tr>
</thead>
</table>
| Transient ND | C  Mitral valve insufficiency, thyroid insufficiency, β thalassaemia  
| E*  Aortic arch defect, multiple epiphyseal dysplasia, high blood pressure  
| J  Multiple epiphyseal dysplasia  
| K  Left kidney agenesis, brain atrophy |
| Permanent ND | D  Lung dysplasia, high blood pressure  
| G  Hypogonadism  
| I3*  Thyroid agenesis, pancreas dysplasia, hepatic failure, kidney failure, high blood pressure  
| I4*  Thyroid hypoplasia, pancreas dysplasia, multiple epiphyseal dysplasia, hepatic failure, kidney failure |

*Case report in Zeller et al.

DISCUSSION

Transmission of the chromosome 6 microsatellite marker was studied for the 14 ND patients. A chromosome 6 paternal iso-

Table 2 Phenotypes and developmental abnormalities of patients with neonatal diabetes (ND)

<table>
<thead>
<tr>
<th>Patients</th>
<th>Phenotypes</th>
</tr>
</thead>
</table>
| Transient ND | C  Mitral valve insufficiency, thyroid insufficiency, β thalassaemia  
| E*  Aortic arch defect, multiple epiphyseal dysplasia, high blood pressure  
| J  Multiple epiphyseal dysplasia  
| K  Left kidney agenesis, brain atrophy |
| Permanent ND | D  Lung dysplasia, high blood pressure  
| G  Hypogonadism  
| I3*  Thyroid agenesis, pancreas dysplasia, hepatic failure, kidney failure, high blood pressure  
| I4*  Thyroid hypoplasia, pancreas dysplasia, multiple epiphyseal dysplasia, hepatic failure, kidney failure |

*Case report in Zeller et al.

a partial paternal uniparental disomy in the transient ND candidate region (not shown).

Two familial permanent ND cases were analysed by haplotyping chromosome 6 microsatellite markers. Family I was consanguineous; a meiotic recombination occurred in patients I3 and I4 between D6S255 and D6S305 on the maternal chromosome 6 and between D6S286 and D6S287 on the paternal chromosome 6, respectively (fig 2). The genotypes for family O are shown in fig 2. In each family, the affected patients inherited different haplotypes of the transient ND candidate region. Multipoint linkage analyses were performed using the MLINK component of the LINKAGE package version 5.1 and the VITESSE algorithm for pedigrees I and O (not shown). Two point linkage analysis gave a maximum lod score of ~4 at θ = 0 for marker D6S409, ~2.10 at θ = 0.001, and ~4.70 at θ = 0.0 for multipoint calculation in pedigree I. In pedigree O, lod scores were ~4.70 at θ = 0.0 multipoint calculation and ~4.70 at θ = 0.0 for marker D6S308. All lod scores calculated in the interval were negative (markers used for pedigree O: D6S1699, D6S314, D6S1684, D6S310, D6S409, D6S308, D6S1003, and D6S311 (data not shown). Both haplotype reconstruction and negative lod score values suggested that the locus on chromosome 6q24 was not linked to the disease in families I and O.

Developmental abnormalities in ND patients

Table 2 shows the congenital abnormalities present in eight of 14 cases in both transient and permanent ND forms. Three patients had thyroid abnormalities: thyroid agenesis in case I4 and hypothyroidism in cases C and I3. Skeletal abnormalities were also observed: multiple epiphyseal and metaphyseal dysplasia in case E and retardation of skeletal maturation in three patients, E, I4, and J. Congenital heart defects were present in patient C who had valvar mitral insufficiency and patient E who had an incomplete aortic arch interruption. Patient K had right kidney agenesis and brain atrophy.

Table 2 Phenotypes and developmental abnormalities of patients with neonatal diabetes (ND)

<table>
<thead>
<tr>
<th>Patients</th>
<th>Phenotypes</th>
</tr>
</thead>
</table>
| Transient ND | C  Mitral valve insufficiency, thyroid insufficiency, β thalassaemia  
| E*  Aortic arch defect, multiple epiphyseal dysplasia, high blood pressure  
| J  Multiple epiphyseal dysplasia  
| K  Left kidney agenesis, brain atrophy |
| Permanent ND | D  Lung dysplasia, high blood pressure  
| G  Hypogonadism  
| I3*  Thyroid agenesis, pancreas dysplasia, hepatic failure, kidney failure, high blood pressure  
| I4*  Thyroid hypoplasia, pancreas dysplasia, multiple epiphyseal dysplasia, hepatic failure, kidney failure |

*Case report in Zeller et al.
IDDM5 (6q25), and IDDM8 (6q27) as well as the candidate locus for the transient ND form at 6q24. None of the five patients with permanent ND had abnormal chromosome 6 marker inheritance. The data support the concept of a different aetiology for transient and permanent ND forms.

Associations of developmental abnormalities that have not been previously highlighted occurred in eight of the 14 (57%) ND patients, affecting 4/9 transient ND and 4/5 permanent ND. As the mothers of the patients were not exposed to drugs or chemicals and were not overtly diabetic during pregnancy, the condition is probably not coincidental and is more likely to be the result of innate errors of development, probably of genetic origin. The classical features of transient ND include macroglossia* and umbilical hernia in 2/30 transient ND.16

Table 3  Review of published reports of congenital abnormalities in patients with neonatal diabetes (ND)

<table>
<thead>
<tr>
<th>ND form/phenotypes</th>
<th>Genetic defect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transient</td>
<td>Macroglossia*</td>
<td>Dacou Youtekakis et al*8</td>
</tr>
<tr>
<td></td>
<td>Macroglossia, umbilical hernia*</td>
<td>Temple et al*8</td>
</tr>
<tr>
<td></td>
<td>Macroglossia, coarse facial features</td>
<td>Hermann et al*5</td>
</tr>
<tr>
<td></td>
<td>Pancreatic β cell agenesis, methylnolic acidemia</td>
<td>Temple et al*8</td>
</tr>
<tr>
<td></td>
<td>Microcephaly</td>
<td>Shield et al*8</td>
</tr>
<tr>
<td></td>
<td>Macroglossia, anemia, umbilical hernia</td>
<td>Temple et al*8</td>
</tr>
<tr>
<td></td>
<td>Macroglossia, hypotelorism, club foot</td>
<td>Christian et al*11</td>
</tr>
<tr>
<td></td>
<td>Macroglossia, umbilical hernia, bilateral inguinal hernia, asymmetrical growth retardation, large fontanelles, hypospadias</td>
<td>Batin et al*10</td>
</tr>
<tr>
<td></td>
<td>Umbilical hernia, delayed development, minor facial anomalies including &quot;carp mouth&quot;, cardiomegaly</td>
<td>Zneimer et al*2</td>
</tr>
<tr>
<td>Permanent</td>
<td>Pancreatic hypoplasia, congenital heart defect</td>
<td>Yorifuji et al*8</td>
</tr>
<tr>
<td></td>
<td>(transposition of the great vessels, ventricular septal defect, pulmonary stenosis, atrial septal defect), microcephaly, large brain mass</td>
<td>Gurson et al*8</td>
</tr>
<tr>
<td></td>
<td>Dorsal pancreas agenesis, interventricular septal defect</td>
<td>Reus et al*8</td>
</tr>
<tr>
<td></td>
<td>Type 1 diabetes, pancreas hypoplasia</td>
<td>Carroll et al*8</td>
</tr>
<tr>
<td></td>
<td>Microcephaly</td>
<td>Wright et al*8</td>
</tr>
<tr>
<td></td>
<td>Pancreatic exocrine insufficiency, congenital pancreatic agenesis</td>
<td>Mitochondrial diabetes eliminated</td>
</tr>
<tr>
<td></td>
<td>Primary congenital hypothyroidism</td>
<td>Muina et al*3</td>
</tr>
<tr>
<td></td>
<td>Hypothyroidism, bilateral neurosensory deafness, myopia, dysmorphic features, congenital stridor, growth retardation</td>
<td>Al Jarayyan et al*2</td>
</tr>
<tr>
<td></td>
<td>Pancreatic agenesis</td>
<td>Mitochondrial diabetes eliminated</td>
</tr>
<tr>
<td></td>
<td>Renal hepatic pancreatic dysplasia</td>
<td>Stoffers et al*3</td>
</tr>
<tr>
<td></td>
<td>Cerebella agenesis/hypoplasia</td>
<td>Hoveyda et al*3</td>
</tr>
</tbody>
</table>

nd=not determined.

*Macroglossia in 5/30 transient ND, association of macroglossia and umbilical hernia in 2/30 transient ND.16

Variants of ND responsible genes and epigenetic determinants involved in transient ND may be key contributors to suboptimal pancreatic development which later predisposes the child to long term abnormalities in insulin production, obesity, and metabolic disturbances or common complex diseases arising with age, such as T2D. The elucidation of genetic and molecular mechanisms in rare neonatal diabetes may provide evidence for new pathways connecting development and glucose metabolism with further insight into consequences in later life.

- Fourteen patients with transient or permanent ND were analysed at the molecular level and their main clinical features were recorded and integrated into a data collection of previously published case reports.
- Two of nine infants with transient ND displayed paternal chromosome 6 isodisomy. Haplotype analysis in two familial permanent ND cases were consistent with the non-involvement of the 6q24 candidate transient ND locus.
- Birth defects were observed in eight of 14 (57%) ND patients with different associations. Macroglossia and umbilical hernia were exclusively associated with the transient ND form. In permanent ND, associations of thyroid, pancreas, liver, heart, kidney, and skeletal abnormalities were found.
- Alterations in genes essential for glucose metabolism may lead to early onset insulin dependent diabetes mellitus with non-random patterns of early embryonic developmental abnormalities. Assigning patients to subgroups for further genetic analysis may help to unravel genetic and molecular mechanisms leading to these phenotypes.
ACKNOWLEDGEMENTS

We would like to thank the patients and their relatives who provided samples for our analysis and the clinicians who referred their patients. Catherine Turleau for critical reading of the manuscript, Mathilde Varret and Jean Pierre Rabès for genetic linkage analysis and helpful discussion, Cécile Julier and Marc Délepine for discussion and the EIF2AK3 gene sequence. This work was supported by the Centre National de la Recherche Scientifique and by grants from the Foundation of France and the Institut National de la Santé et de la Recherche Médicale. EM is the holder of a student fellowship from the French Ministère de l’Éducation Nationale de la Recherche et de la Technologie.

...............

Authors’ affiliations

E Marquis, C Junien, C Diatloff-Zito, INSERM U383, Hôpital Necker-Enfants Malades, 149-161 rue de Sèvres, 75743 Paris Cedex 15, France

J J Robert, Fédération de Pédiatrie, Hôpital Necker-Enfants Malades, Université de Paris V, 149-161 rue de Sèvres, 75743 Paris Cedex 15, France

C Bouvattier, Hôpital Saint Vincent de Paul, Paris, France

C Bellanné-Chantelot, Laboratoire de Génétique Médicale, Fondation Jean Dausset CEPH, Paris, France

Correspondence to: Dr C Diatloff-Zito, INSERM U383, Hôpital Necker-Enfants Malades, 149-161 rue de Sèvres, 75743 Paris Cedex 15, France; diatloff@necker.fr

REFERENCES


23 Byberg L, McKiegue PM, Zethelius B, Lithell HO. Birth weight and insulin resistance syndrome: association of low birth weight with truncal obesity and raised plasmagamine activator inhibitor 1 but not with abdominal obesity or plasma lipid disturbances. Diabetologia 2000; 43:54-60.

Major difference in aetiology and phenotypic abnormalities between transient and permanent neonatal diabetes

E Marquis, J J Robert, C Bouvattier, C Bellanné-Chantelot, C Junien and C Diatloff-Zito

doi: 10.1136/jmg.39.5.370

Updated information and services can be found at:
http://jmg.bmj.com/content/39/5/370

These include:

References
This article cites 37 articles, 7 of which you can access for free at:
http://jmg.bmj.com/content/39/5/370#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

Diabetes (105)
Metabolic disorders (329)
Calcium and bone (307)
Immunology (including allergy) (604)
Molecular genetics (1254)
Genetic screening / counselling (887)
Clinical diagnostic tests (356)
Ethics (220)
Reproductive medicine (519)
Pancreas and biliary tract (110)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/