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

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LETTER TO JMG

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, ZAC/PLAGL1 (zinc finger, apoptosis, cell cycle/pleomorphic adenoma of the salivary gland gene like 1) and HYMAI (hydatiform 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) 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 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) |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Clinical features | Patients | A | B | C | D | E | F | G | I3 | I4 | J | K | M* | N | O | |
| Sex | F | F | F | M | F | M | M | F | M | M | F | M | M | F |
| Birth weight (g) | 1670 2190 2800 2600 1750 1960 | na | 1900 | 2020 | 1840 | 3200 | 1600 | 3160 | 3050 |
| Age at diagnosis | 13 d 5 d | 2 d 1 d | na | 5 d 1 d | 1 d 1 d | 26 d 6 d | na | 6 mth |
| Diabetes form | Tr | Tr | Tr | P | Tr | P | P | Tr | Tr | Tr | Tr | P |
| Insulin† | 12 d 5 mth | 2.5 mth 4.5 mth | 4.5 mth | 13 mth | 5 mth | na |
| Family diabetes history | – | – | T1D | – | – | – | – | T1D | T1D | – | – | T1D | T1D | – | – |
| Congenital birth anomalies§ | – | – | ++ | ++ | ++ | ++ | ++ | T2D | T2D | T2D | yes‡ |

†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.
‡Case O7 is subject O7 of a multiplex family with insulin dependent diabetes appearing before 6 months of age (see pedigree in fig 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
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 algorithm11 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 I3 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.

**DISCUSSION**

Transmission of the chromosome 6 microsatellite marker was studied for the 14 ND patients. A chromosome 6 paternal isodisomy was identified in 2/9 patients with transient ND, an abnormality which accounts for 20 to 30% of transient ND cases. Biparental transmission of the chromosome 6 alleles was found in seven out of nine patients with transient ND. In some of these patients, the disease is expected to result from an altered epigenotype. Methylation defects at the unique differentially methylated CpG island have been found in 47% of the patients examined: (2/8), (9/20), (5/6). However, no mutation at the ZAC1/PLAGL1 gene locus could be identified in the transient ND patients examined.

Haplotype analysis in two familial permanent ND cases showed the lack of a common haplotype in affected subjects and a negative lod score excluded the involvement of the loci predisposing to T1D on chromosome 6: IDDM15 (6q21),
Table 3  Review of published reports of congenital abnormalities in patients with neonatal diabetes (ND)

<table>
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<tr>
<th>ND form/phenotypes</th>
<th>Genetic defect</th>
<th>References</th>
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</thead>
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<tr>
<td><strong>Transient</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macroglossia*</td>
<td>nd</td>
<td>Dacou Youtekakis et al4</td>
</tr>
<tr>
<td>Macroglossia, umbilical hernia*</td>
<td>Paternal UPD(6)</td>
<td>Temple et al37</td>
</tr>
<tr>
<td>Macroglossia, coarse facial features</td>
<td>Paternal inv dup (6)(q22q23)</td>
<td>Arthur et al13</td>
</tr>
<tr>
<td>Pancreatic β cell agenesis, methylmalonic acidemia</td>
<td>Paternal UPD(6)</td>
<td>Abramovitz et al31</td>
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<tr>
<td>Microcephaly</td>
<td>nd</td>
<td>Shield et al3</td>
</tr>
<tr>
<td>Macroglossia, anaemia, umbilical hernia</td>
<td>Paternal dup(6)</td>
<td>Temple et al3</td>
</tr>
<tr>
<td>Macroglossia, hypertelorism, club foot</td>
<td>Paternal UPD(6)</td>
<td>Christian et al1</td>
</tr>
<tr>
<td>Macroglossia, umbilical hernia, bilateral inguinal hernia, asymmetrical growth retardation, large fontanelles, hypospadias</td>
<td>nd</td>
<td>Battin et al39</td>
</tr>
<tr>
<td>Umbilical hernia, delayed development, minor facial anomalies including “carp mouth”, cardiomegaly</td>
<td>Dup(6)(q21q23)</td>
<td>Zneimer et al32</td>
</tr>
<tr>
<td><strong>Permanent</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreatic hypoplasia, congenital heart defect (transposition of the great vessels, ventricular septal defect, pulmonary stenosis, atrial septal defect), neocrotic brain mass</td>
<td>nd</td>
<td>Yorifuji et al34</td>
</tr>
<tr>
<td>Dorsal pancreas agenesis, interventricular septal defect</td>
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<td>Type 1 diabetes, pancreas hypoplasia</td>
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<td>Carroll et al36</td>
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<td>Primary congenital hypothyroidism</td>
<td>nd</td>
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<tr>
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<td>Mitochondrial diabetes eliminated</td>
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<tr>
<td>Pancreatic agenesis</td>
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<td>Renal hepatic pancreatic dysplasia</td>
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<tr>
<td>Cerebellar agenesis/hypoplasia</td>
<td>nd</td>
<td>Hoveyda et al43</td>
</tr>
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</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.

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 macroglossia13 and umbilical hernia4 16 19–22 (table 3). Other defects arising in the patients in this report show a larger than expected number of congenital abnormalities in transient ND17 that may be related to the nature of the genetic and epigenetic alterations involved.12 13 Congenital malformations in patients with permanent ND are different from those of transient ND with combinations including organs derived from branching to the main visceral tube26 (table 3). The nature of the associated birth defects in ND patients suggests that the events responsible occurred during the early stages of embryogenesis.15 16 Three patients, cases E, J, and 14, had birth defect associations overlapping those of some patients with the Volcott-Rallison syndrome (OMIM 226980).26 27 Although the EIF2AK3 gene was not mutated in these three patients (not shown), this raises the possibility of functional pathway interactions. It is therefore important to assess critically currently held concepts of pathological development within carefully defined ND patient subgroups.

Low birth weight is a classical feature of ND patients. This condition is known to be associated with a later risk of dyslipidaemia, hypertension, cardiovascular disease,36 and T2D.37

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