Original Article

Mutations in ATP-Sensitive K^+ Channel Genes Cause Transient Neonatal Diabetes and Permanent Diabetes in Childhood or Adulthood

Sarah E. Flanagan,[1] Ann-Marie Patch,[1] Deborah J.G. Mackay,[2,3] Emma L. Edghill,[1] Anna L. Gloyn,[1,4] David Robinson,[2] Julian P.H. Shield,[5] Karen Temple,[3,6] Sian Ellard,[1] and Andrew T. Hattersley[1]

Transient neonatal diabetes mellitus (TNDM) is diagnosed in the first 6 months of life, with remission in infancy or early childhood. For ~50% of patients, their diabetes will relapse in later life. The majority of cases result from anomalies of the imprinted region on chromosome 6q24, and 14 patients with ATP-sensitive K^+ channel (K_{ATP} channel) gene mutations have been reported. We determined the 6q24 status in 97 patients with TNDM. In patients in whom no abnormality was identified, the *KCNJ11* gene and/or *ABCC8* gene, which encode the Kir6.2 and SUR1 subunits of the pancreatic β-cell K_{ATP} channel, were sequenced. K_{ATP} channel mutations were found in 25 of 97 (26%) TNDM probands (12 *KCNJ11* and 13 *ABCC8*), while 69 of 97 (71%) had chromosome 6q24 abnormalities. The phenotype associated with *KCNJ11* and *ABCC8* mutations was similar but markedly different from 6q24 patients who had a lower birth weight and who were diagnosed and remitted earlier (all $P < 0.001$). K_{ATP} channel mutations were identified in 26 additional family members, 17 of whom had diabetes. Of 42 diabetic patients, 91% diagnosed before 6 months remitted, but those diagnosed after 6 months had permanent diabetes ($P < 0.0001$). K_{ATP} channel mutations account for 89% of patients with non-6q24 TNDM and result in a discrete clinical subtype that includes biphasic diabetes that can be treated with sulfonylureas. Remitting neonatal diabetes was observed in two of three mutation carriers, and permanent diabetes occurred after 6 months of age in subjects without an initial diagnosis of neonatal diabetes. *Diabetes* 56:1930–1937, 2007

From the [1]Institute of Biomedical and Clinical Science, Peninsula Medical School, Exeter, U.K.; the [2]Wessex Regional Genetics Laboratories, Salisbury District Hospital, Salisbury, U.K.; the [3]Division of Human Genetics, Southampton University, Southampton, U.K.; the [4]Diabetes Research Laboratories, Oxford Centre for Diabetes Endocrinology and Metabolism, University of Oxford, Oxford, U.K.; [5]The Royal Hospital for Children, Bristol, U.K.; and the [6]Wessex Clinical Genetics Service, National Health Service Trust, Southampton, U.K.

Address correspondence and reprint requests to Professor Andrew T. Hattersley, Peninsula Medical School, Barrack Road, Exeter, EX2 5DW, U.K. E-mail: a.t.hattersley@exeter.ac.uk.

Received for publication 15 January 2007 and accepted in revised form 6 April 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 19 April 2007. DOI: 10.2337/db07-0043.

K_{ATP} channel, ATP-sensitive K^+ channel; PNDM, permanent neonatal diabetes mellitus; TNDM, transient neonatal diabetes mellitus.

Transient neonatal diabetes mellitus (TNDM) is a clinically defined subgroup affecting ~50% of children with neonatal diabetes. TNDM is differentiated from permanent neonatal diabetes mellitus (PNDM) because the diabetes remits in infancy or early childhood. Relapse in childhood or adolescence occurs in up to 50% of cases (1).

There has been considerable progress in defining the genetic etiology of TNDM. The major breakthrough was establishing that the majority of cases result from anomalies of the imprinted region on chromosome 6q24, which encodes the *ZAC* and *HYMAI* genes (2,3). Three types of abnormalities have been identified to date that result in the overexpression of the paternal allele: paternally inherited duplication of chromosome 6q24, paternal uniparental isodisomy of chromosome 6, or a methylation defect.

Recent studies have identified 14 patients with TNDM resulting from mutations in the *KCNJ11* and *ABCC8* genes that encode the Kir6.2 and SUR1 subunits of the potassium ATP-sensitive K^+ channel (K_{ATP} channel) in the pancreatic β-cell (4–6,8). Heterozygous activating mutations in the *KCNJ11* gene are the commonest cause of *PNDM* but also have been described in five patients with TNDM (4–6). Functional work illustrated that TNDM mutations were functionally less severe in vitro than mutations resulting in PNDM (4). Activating heterozygous *ABCC8* mutations have recently been reported in three patients with PNDM and seven with TNDM (7,8). Mutations in either gene encoding the subunits of the K_{ATP} channel cause diabetes by reducing the ability of the channel to close in response to increased ATP and reduced ADP concentrations. This altered sensitivity of the channel to the ratio of ATP to ADP results in increased potassium efflux from the cell and hyperpolarization of the membrane, thus reducing insulin secretion from the β-cell. The mechanism by which K_{ATP} channel mutations result in a remitting/relapsing diabetes phenotype is not known.

The prevalence of K_{ATP} channel mutations in TNDM and the associated clinical characteristics are uncertain. The initial descriptions suggest that the onset of diabetes and remission is later in TNDM patients with K_{ATP} channel mutations (1,4). Since the original criteria for TNDM are based on a series of patients with 6q24 diabetes (diabetes diagnosed within 6 weeks of birth with remission by 18 months) (9), this definition may exclude some cases with K_{ATP} channel mutations. We and others (10,11) have shown that type 1 diabetes is very rare before 6 months of

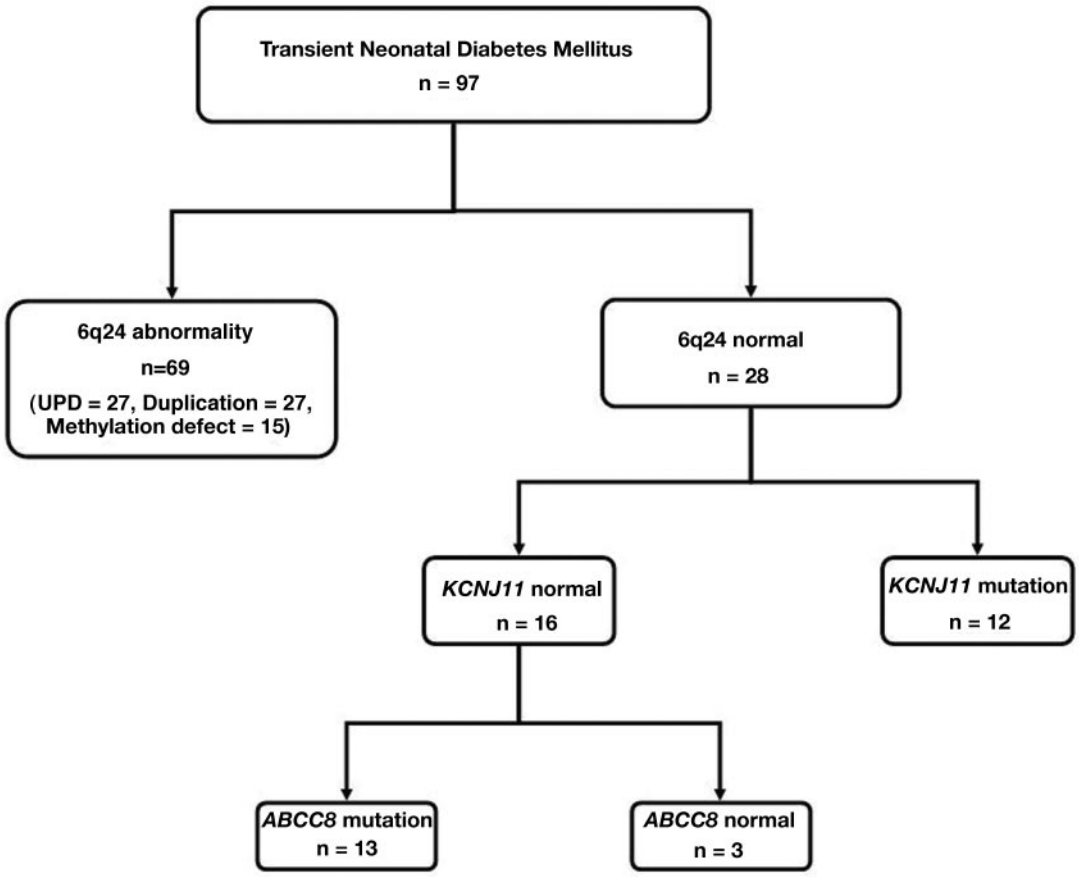

FIG. 1. Number of patients diagnosed with TNDM and results of genetic testing. UPD, uniparental isodisomy.

age and suggests a genetic etiology for most patients diagnosed before 6 months. TNDM might therefore be redefined as diabetes diagnosed before 6 months of age that later remits.

We investigated the genetic etiology of 97 patients whose diabetes was diagnosed within the first 6 months of life but entered remission before age 5 years. Our aim was to assess the prevalence of 6q24 abnormalities and K_{ATP} channel mutations within this cohort and to investigate the clinical features of mutation carriers.

RESEARCH DESIGN AND METHODS

We studied 97 patients with diabetes diagnosed in the first 6 months of life whose diabetes remitted before the age of 5 years. Sixty-four have been previously reported (1,3,4,12–15). The patients were referred from 14 different countries across four continents and were either recruited following a request for referrals to the International Society of Pediatric and Adolescent Diabetes rare diabetes collection or following referral to the Wessex Regional Genetics Laboratory for 6q24 abnormality testing. The study was conducted in accordance with the Declaration of Helsinki, as revised in 2000. Informed consent was obtained from all patients, with parental consent given on behalf of children.

Genetic analysis. Genomic DNA was extracted from peripheral lymphocytes using standard procedures. In all patients, analysis of the 6q24 locus was undertaken using previously described methods to detect duplications, uniparental isodisomy and methylation abnormalities (1,13). In patients for whom no 6q24 abnormality was identified, the single exon of *KCNJ11* was amplified in three overlapping fragments, as previously described (16). The 39 exons of *ABCC8* were analyzed in all patients in whom no *KCNJ11* mutation was identified. The *ABCC8* gene was amplified in 38 fragments using previously described primers (7). PCR products were sequenced using standard methods on an ABI 3100 or ABI 3730 (Applied Biosystems, Warrington, U.K.). Sequences were compared with the published sequences ([*KCNJ11* – NM_000525.3] [*ABCC8* – NM_000352.2]) using Staden analysis or Mutation Surveyor version 2.61. Mutations were tested for cosegregation with diabetes in other family members and in 200 normal chromosomes from U.K. Caucasians. Where possible, family relationships were confirmed using a panel of six microsatellite markers on chromosome 11p (16). Clinical characteristics were obtained from the patient's hospital records with assistance from their physician. Clinical characteristics are presented as median (range), and comparative statistics predominantly used the Mann-Whitney *U* test.

RESULTS

Prevalence of different genetic aetiologies. We have determined the genetic etiology in 94 of 97 (97%) probands diagnosed with diabetes before the age of 6 months whose diabetes had subsequently entered remission (Fig. 1). The majority of patients (69 of 97) had an abnormality at the 6q24 locus. Of these patients, 27 (39%) had paternal uniparental isodisomy, 27 (39%) had a duplication of the paternal allele, and 15 (22%) had a methylation defect. For the 28 remaining patients, in whom no 6q24 aberration was identified, sequencing of *KCNJ11* and *ABCC8* demonstrated that 25 probands were heterozygous for K_{ATP} channel mutations.

Mutation characteristics

KCNJ11 mutations. Twelve of the probands had a heterozygous mutation in the *KCNJ11* gene; six of these patients (with five different mutations) have been reported previously (4,12). We found eight different *KCNJ11* mutations; R34C (c.100C>T), G53R (c.157G>C), G53S (c.157G>A), E179A (c.536A>C), I182V (c.544A>G), E227K (c.679G>A), E229K (c.685G>A), and R365H (c.1094G>A). Two mutations were identified in more than one proband (E227K, $n = 2$, and E229K, $n = 4$). The

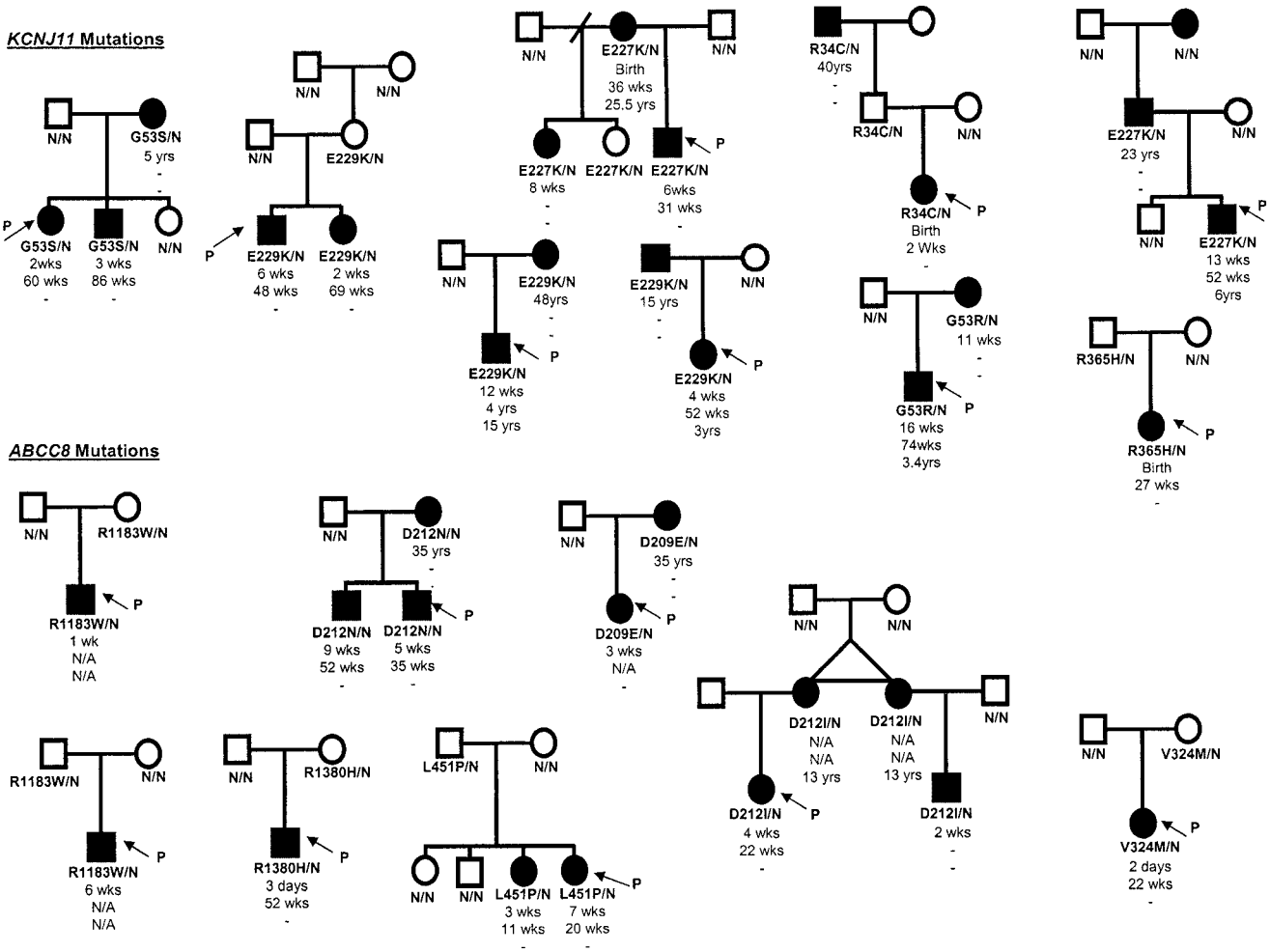

FIG. 2. Partial pedigrees of families showing inheritance of *KCNJ11* or *ABCC8* mutations. Squares represent male family members, and circles represent female subjects. Filled symbols denote patients with diabetes. Genotype is shown underneath each symbol, residue number and amino acid change are given for the mutation carriers, and N/N denotes no mutation identified. Directly below the genotype is the age of initial diagnosis for the mutation carriers, followed by the age of remission and the age of relapse. A dash represents a nonevent, and N/A denotes information not available. An arrow with the letter P points to the proband in each family.

mutations R34C, E179A, and R365H are novel. We did not observe these mutations in 200 normal chromosomes, and all mutations affect residues that are conserved in human, mouse, rat, and dog (http://genome.ucsc.edu).

ABCC8 mutations. Ten different *ABCC8* gene mutations were identified in 13 probands: D209E (c.627C>A), D212N (c.634G>A), D212I (c.634 G>A 635A>T), V324M (c.970G>A), L451P (c.1352T>C), R826W (c.2476C>T), R1183W (c.3547C>T), R1183Q (c.3548G>A), R1380C (c.4138C>T), and R1380H (c.4139G>A). All were identified in one proband, with the exception of R1183W, which was identified in four probands. All mutations were novel except for R1380C and R1183Q (8). We did not observe these mutations in 200 normal chromosomes, and all affect residues that are conserved in human, mouse, rat, and dog (http://genome.ucsc.edu).

Inheritance of mutations. Seven (28%) probands had de novo mutations as confirmed by the absence of the mutation in the unaffected parents (*KCNJ11*, $n = 3$, and *ABCC8*, $n = 4$). Microsatellite analysis was used to verify the family relationships in all but one case with an R1380C *ABCC8* mutation, in whom parental DNA was unavailable. In the remaining 17 probands, the mutation had been inherited from a parent (partial pedigrees are shown in Fig. 2). In these families, 17 of 26 parents or siblings with the K_{ATP} channel mutation had diabetes; in contrast, only 1 family member without the mutation was affected ($P < 0.001$). However there was not complete cosegregation of the mutations with diabetes because in eight probands the mutation was inherited from a nondiabetic parent. Neonatal diabetes (diagnosed in the first 6 months) was described in five siblings and four parents. Two mothers (identical twins, Fig. 2) were reported to have had diabetes in the neonatal period, although information regarding their ages at diagnosis and remission was not available. Nonpenetrance was seen in families with eight different mutations, suggesting that it was not a feature of specific mutations.

Clinical characteristics of probands with *KCNJ11* or ABCC8 mutations

Diabetes. The characteristics of diabetes in the probands with *KCNJ11* or *ABCC8* mutations are shown in Table 1. There was no significant difference in clinical characteristics between these two groups. Reduced birth weight was a consistent feature of both *KCNJ11* and *ABCC8* mutations (25th vs. 11th percentile; $P = 0.26$). Diabetes was most frequently diagnosed between 0 and 8 weeks (86%), with all cases diagnosed before 17 weeks. None of the patients with an *ABCC8* mutation had relapsed at the time of this study; however, this may be due to the younger age

TABLE 1
Clinical characteristics of probands with an unknown genetic etiology and patients with a K_{ATP} channel mutation

Characteristic	Genetic etiology unknown	*KCNJ11*	*ABCC8*	Comparison of *KCNJ11* and *ABCC8* mutation carriers (*P* value)
n (% male)	3 (100)	12 (58)	13 (54)	0.33
Age at diagnosis (weeks)	3 (1–4)	5 (0–16)	4 (0–9)	0.53
Age at remission (weeks)	8 (17–208)	45 (2–208)	22 (7–52)	0.14
Age when entering study (years)	14 (1–31)	7.5 (0.84–17)	5 (0.84–16)	—
Diabetes relapsed (*n*)	2	4	0	—
Age at relapse (years)	11 (5–17)	4.7 (3–15)	—	—
Gestation (weeks)	38 (30–40)	38 (30–40)	39 (30–41)	0.17
Birth weight (g)	1,620 (1,100–3,373)	2,570 (1,535–3,570)	2,575 (1,360–3,400)	0.73
Centile birth weight	6 (<1st to 35th)	25 (<1st to 89th)	11 (<1st to 32nd)	0.26

Data are median (range), unless otherwise indicated. Comparison of clinical characteristics of patients with *KCNJ11* mutation and *ABCC8* mutation. Differences between groups were calculated using Mann-Whitney *U* and χ^2 tests. Centile birth weights were calculated according to U.K. growth charts (27) because the majority of patients were of U.K. white origin.

of these patients compared with those with *KCNJ11* mutations.

Neurological features. In addition to diabetes, neurological features were identified in four probands (16%) and were associated with both *KCNJ11* and *ABCC8* mutations. Two probands and one family member with a *KCNJ11* mutation had neurological features. The first proband with an E229K *KCNJ11* mutation has reported speech delay and was diagnosed with autistic spectrum disorder at the age of 3 years. Interestingly, the affected sibling and unaffected mother who both carry the same mutation have no reported speech problems, although the sibling is currently 2 years of age and still in the early stages of language development. The E229K mutation has been identified in five affected patients from three further families, with no reports of speech or developmental delay in any of the mutation carriers. Mild learning difficulties were observed in a second proband and his mother who both have the G53R *KCNJ11* mutation. This mutation has not been identified in any other families to date; therefore, it is difficult to conclude whether these complications are a feature of the mutation or whether environmental and/or other genetic factors are involved.

Neurological features were also reported in three patients with an *ABCC8* mutation. Muscle weakness was identified in two cousins with a *D212I* mutation. The proband had muscle hypotonia until the age of 8 months, and her cousin has been diagnosed with motor developmental delay, although no muscle weakness was reported in either of their affected mothers. The most severe neurological phenotype was identified in a proband with a de novo *ABCC8* mutation (R1183W). This patient had one episode of tonic posturing with right facial involvement following admission to the hospital with diabetic ketoacidosis. He subsequently had two episodes of generalized seizures shortly after diagnosis of diabetes. No further seizures have been reported since insulin therapy was started, and it is likely that these seizures were a result of the severe hyperglycemia rather than as part of the DEND (Developmental Delay, Epilepsy, and Neonatal Diabetes) syndrome (17). Neurological features have not been reported in any of the five other patients with the R1183W mutation.

The clinical characteristics of the probands with K_{ATP} channel mutations were markedly different when compared with subjects with 6q24 abnormalities (Table 2). Patients with a 6q24 abnormality presented with a more severe clinical phenotype, as shown by their lower percentile birth weight (<1st vs. 12th; $P < 0.001$) and earlier age of diagnosis (0 vs. 4 weeks; $P < 0.001$). For patients with a K_{ATP} channel mutation, the initial episode of diabetes was longer, with diabetes remitting significantly later than in patients with a 6q24 abnormality (35 vs. 13 weeks; $P < 0.001$). There is a trend toward an earlier age of relapse in patients with a K_{ATP} channel mutation ($P = 0.07$), and this may become more evident as the patients are studied over a longer period of time.

Clinical characteristics of the K_{ATP} channel mutations vary according to the age of diagnosis of initial diabetes. The characteristics of the diabetes varied according to the age at which it was first diagnosed (see Table 3). Remission of diabetes occurred in 32 of 35 (91%) patients diagnosed before 6 months but in none of those diagnosed after 6 months ($P < 0.001$) (Table 3 and Fig. 3). The median age at remission was 35 weeks (range 2–208). The three patients diagnosed before 6 months who had not entered remission were 1, 2, and 43 years of age at mutation screening.

All patients were treated with insulin during their initial episode of diabetes and during any subsequent relapse of diabetes, except for one proband and his father with a *KCNJ11* mutation (E227K), a father with a *KCNJ11* mutation (E229K), and a mother with an *ABCC8* mutation (D209E), who were treated with sulfonylureas. Two probands and four family members have now attempted to transfer off insulin and onto sulfonylureas using a protocol similar to that described for *KCNJ11* mutations (18). Transfer was successful in five cases (83%) but unsuccessful in a mother with a G53R mutation. Her son, who carries the same mutation, has successfully transferred onto sulfonylureas, suggesting that the observed lack of response to sulfonylureas is not a feature of the mutation.

Phenotype of patients in whom the genetic etiology is not defined. We were able to determine the genetic etiology in all but 3 of 97 probands diagnosed with TNDM. Interestingly, all three patients had an atypical phenotype. The first patient was born at 30 weeks' gestation, and his transient hyperglycemia was possibly a consequence of

TABLE 2
Clinical characteristics of probands grouped by genetic etiology

Characteristic	Combined *KCNJ11* and *ABCC8*	6q24*	Comparison of *KCNJ11 and ABCC8* combined data and 6q24 patients (*P* value)
n (% male)	25 (56)	23 (53)	0.94
Age at diagnosis (weeks)	4 (0–16)	0 (0–4)	<0.001
Age at remission (weeks)	35 (2–208)	13 (5–60)	<0.001
jAge when entering study (years)	6 (0.84–17)	12 (1–36)	—
Diabetes relapsed (*n*)	4	7	—
Age at relapse (years)	4.7 (3–15)	16 (4–25)	0.073
Gestation (weeks)	38 (30–41)	40 (36–42)	0.059
Birth weight (g)	2,570 (1,360–3,570)	1,950 (1,600–2,670)	<0.001
Centile birth weight	12 (<1st to 89)	<1st (<1st to 21)	<0.001

Data are median (range), unless otherwise indicated. Comparison of probands with a K_{ATP} channel mutation (combined results) to patients with a 6q24 abnormality. *Data previously reported (1). Differences between groups were calculated using Mann-Whitney *U* and χ^2 tests. Centile birth weights were calculated according to U.K. growth charts (27) because the majority of patients were of U.K. white origin.

the premature birth. Early gestational age was not a consistent feature in patients with either a 6q24 anomaly or a K_{ATP} channel mutation. The second patient stopped insulin treatment at the age of 4 years, but this was recommenced 1 year later. He also developed epilepsy at the age of 9 years, which has not been reported in any

TABLE 3
Comparison of clinical and biochemical characteristics of patients with a K_{ATP} channel mutation diagnosed before 6 months of age with patients whose diabetes was not diagnosed before age 6 months and the number of each mutation identified within each group

Characteristic	Mutation carriers diagnosed with diabetes within 6 months	Mutation carriers who did not have diabetes diagnosed within the first 6 months	*P* value
n (% male)	35 (51)	16 (44)	0.75
Probands (*n*)	25	0	
Age when entering study (years)	6 (0.8–43)	42 (5–56)	—
Ever diagnosed with diabetes (*n*)	35	7	$1*10^{-6}$
Age at diagnosis (weeks)	4 (0–17)	1196 (260 to >2496)	$3.7*10^{-5}$
Diabetes remitted (*n*)	32	0/7	$3.7*10^{-10}$
Age at remission (weeks)	35 (2–208)	—	—
Diabetes relapsed (*n*)	7	—	—
Age at relapse (years)	13 (3–25.5)	—	—
Birth weight (g)	2,695 (1,360–3,570)	2,810 (907–3,090)	0.9
Gestation (weeks)	39 (30–42)	38 (34–40)	0.74
Centile birth weight	18 (<1st to 89th)	15 (<1st to 79th)	0.94
KCNJ11 mutations			
R34C	1	2	
G53R	2	0	
G53S	2	1	
E179A	1	0	
I182V	1	0	
E227K	4	2	
E229K	5	3	
R365H	1	1	
ABCC8 mutations			
D209E	1	1	
D212N	2	1	
D212I	4	0	
V324M	1	1	
L451P	2	1	
R826W	1	0	
R1183W	4	2	
R1183Q	1	0	
R1380C	1	0	
R1380H	1	1	

Data are median (range), unless otherwise indicated. Differences between groups were calculated using Mann-Whitney *U* and χ^2 tests. Percentile birth weights were calculated according to U.K. growth charts (27) because the majority of patients were of U.K. white origin.

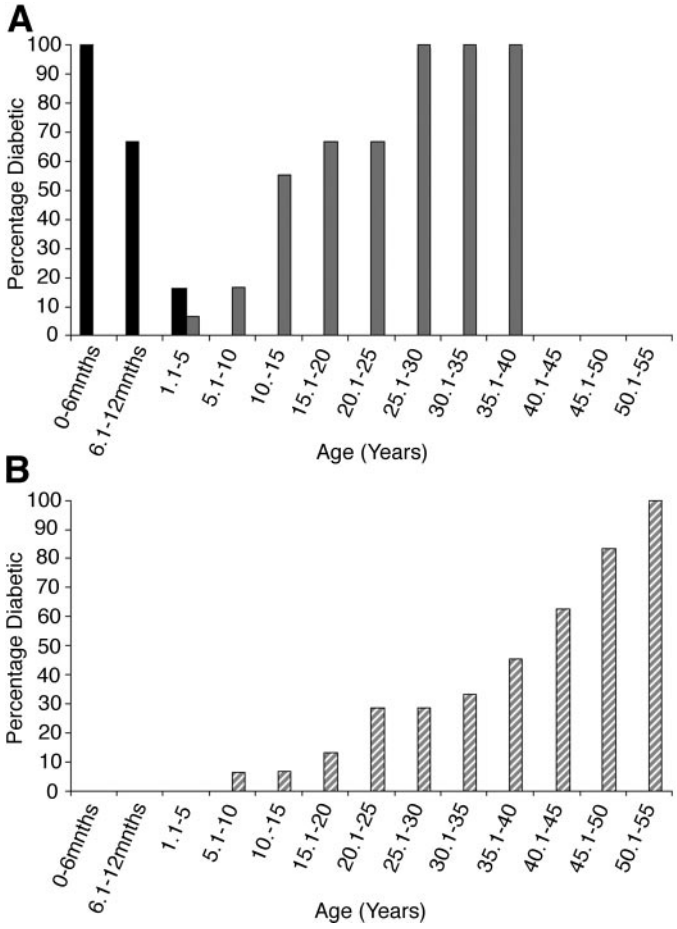

FIG. 3. *A*: Patients diagnosed with diabetes before 6 months of age, which subsequently entered remission. The diagram illustrates the percentage of patients diabetic at any given age. Black columns represent the initial diagnosis, and grey columns represent the second episode of diabetes (relapse). Percentages were calculated according to the number of patients who had reached that given age. *B*: Patients diagnosed with diabetes after 6 months of age. The diagram represents the percentage diabetic at any given age. Percentages were calculated according to the number of patients who had reached that given age.

other patients in our series. The third patient has had four episodes of remission and relapse, a unique phenotype that has also not been described in any other patients included in this study. In addition, the coding sequence of the glucokinase gene was normal in all three patients. This phenotypic variation suggests further rare distinct etiologies for TNDM.

DISCUSSION

We have shown that mutations in the *KCNJ11* and *ABCC8* genes that encode the Kir6.2 and SUR1 subunits of the K_{ATP} channel are a major cause of TNDM, accounting for 29% of all cases and 89% of non-6q24 TNDM. As a result, a genetic diagnosis could be made in 97% of patients with neonatal diabetes that remitted. These estimates of the relative prevalence of the three major genetic subgroups are likely to be robust, as this study of a consecutive series of 97 TNDM patients is more than twice the size of the largest previous study. In our series, *KCNJ11* ($n = 12$) and *ABCC8* ($n = 13$) mutations were equally represented, which is in marked contrast to PNDM, for which there are >60 *KCNJ11* mutations and only 3 *ABCC8* mutations reported (7,8). The only previous comprehensive study is from France, which showed that a genetic diagnosis was possible for 33 of 44 patients with TNDM (8). This is in contrast to the current study, in which the genetic etiology remains unknown in only 3 of 97 patients. The reason for this discrepancy is unknown; it could result from differences in the patients studied or in the detection of K_{ATP} channel mutations. *KCNJ11* mutations have now been identified in a total of 18 patients with TNDM (5,6,19). Our study emphasizes that both *KCNJ11* and *ABCC8* mutations are an important cause of TNDM.

Defining mutations. There is strong evidence that the mutations detected in *KCNJ11* or *ABCC8* are the cause of TNDM in these patients. All of the mutations occur at residues that are conserved across multiple species, alter the coding sequence (an essential requirement for an activating channel mutation), and are not found in normal control subjects or patients with PNDM. The strongest evidence for an etiological role is the de novo mutations, as spontaneous mutations are rare, even in genes as large as *ABCC8* (8.5×10^{-5}; 20). However, de novo mutations are only seen for 4 of the 14 novel mutations (29%) in contrast to PNDM, in which 84% of cases represent spontaneous mutations (21). For the majority of cases (68%), the mutation is inherited from a parent, and although cosegregation of TNDM is rare, all cases of TNDM in the families have inherited the mutation. Fifty percent of parents have diabetes diagnosed before 45 years, which is rare in the general population (<0.2%) (calculated from 22) and is not seen in any of the non–mutation-carrying parents. The final evidence that these mutations are pathogenic is that functional studies for all mutations performed to date (G53R, G53S, I182V, E227K, and E229K in *KCNJ11*) have shown altered response to ATP in the presence of magnesium ions (4,8,19). The presence of de novo mutations identified in five probands strongly supports an etiological role for the I182V, E229K, R1380C, and R1183Q mutations. Therefore, these mutations are highly likely to be causative of TNDM, even in the absence of complete cosegregation.

Location and function of mutations. We have identified a number of commonly mutated residues in patients with TNDM within both K_{ATP} channel genes. *KCNJ11* mutations in our TNDM series cluster with residue G53 mutated in 2 of 12 probands and E227 and E229 residues mutated in 6 of 12 probands. Structural analysis has shown that G53 lies in a region linking the ATP binding site to the transmembrane domain (4), whereas E227 and E229 lie distant to the ATP binding site at the interface between Kir6.2 subunits and are thought to influence channel gating (19).

The *ABCC8* mutations are located throughout the SUR1 protein. The residue R1183, which is located at a position involved in joining transmembrane domain 2 to nucleotide binding domain 2, was mutated in five probands with two different residues, tryptophan and glutamine substituting arginine. The second most commonly affected residue was R1380, which is located in nucleotide binding domain 2 and is mutated in two probands. There are a cluster of mutations (D209E, D212N, and D212I) in the intracellular region that links the transmembrane domain with the gatekeeper module (8).

Functional studies of channels with the TNDM-associated *KCNJ11* mutations G53S, G53R, and I182V showed a moderate reduction in activity in the presence of ATP, which is less marked than the reduction seen with PNDM mutations (4). This suggested clear association of the

genotype with functional effect as well as clinical phenotype, but further studies have shown that there is some overlap between the magnitude of the K_{ATP} channel currents in TNDM- and PNDM-associated mutations (19). Furthermore, the V252A and R201H mutations have been described in both patients with PNDM and TNDM (6,19), so the genotype/phenotype correlation is not absolute.

A biphasic course for diabetes in *KCNJ11* and *ABCC8* mutations associated with TNDM. We have shown that patients with K_{ATP} channel mutations, when diagnosed within the neonatal period, have similar clinical characteristics irrespective of whether *KCNJ11* or *ABCC8* is mutated (Table 1). The TNDM probands with K_{ATP} channel mutations show a biphasic course in which diabetes is diagnosed before age 6 months. They then typically go into remission between age 6 and 12 months and are likely to relapse during adolescence or early adulthood. None of the patients diagnosed with diabetes after 6 months of age had a remission of diabetes, even if they had the same mutation as patients who remitted. Interestingly, birth weight, a reflection of insulin-mediated growth and, hence, insulin secretion in utero, is reduced to a similar extent whether the patient is diagnosed before 6 months or not. These findings imply that the K_{ATP} channel mutations have a biphasic course, and patients diagnosed later may have had a period of hyperglycemia that was undetected in the neonatal period. This could explain the apparent phenotypic heterogeneity with the same mutation. Importantly, this means that K_{ATP} channel mutations can present outside the neonatal period, and, if the parents in our families had not had a child with TNDM, they would have been diagnosed as having type 1 or type 2 diabetes. It will be important to test cohorts with a clinical diagnosis of type 1 diabetes to see how prevalent these mutations are, especially because it will have implications for treatment.

The explanation of the biphasic course in both types of K_{ATP} channel mutations is not known. A fixed-channel defect would be expected to produce a fixed phenotype, as seen with K_{ATP} channel mutations causing PNDM. The change seen in glucose tolerance during remission and subsequent relapse likely reflects either changes in insulin requirements or changes in the number of β-cells or both. As insulin levels are very low in nondiabetic neonates (23), this would suggest that the initial diabetes does not reflect increased insulin requirements at this time, although the relapse around puberty may reflect the associated increase in insulin resistance. The biphasic course seen in 6q24 TNDM is still not fully understood, even with the creation of a good mouse model (24).

Neurological complications were present in addition to diabetes in six patients with an *ABCC8* or *KCNJ11* mutation. In all cases, the affected individual had been diagnosed with diabetes before the age of 6 months. In one family, both mutation carriers had learning difficulties. In all other families, neurological features did not cosegregate with the mutation, suggesting that neurological phenotype is not a consistent feature of these mutations.

6q24 TNDM differs from TNDM resulting from K_{ATP} channel mutations. Our series provides strong evidence that clinical characteristics differ markedly between patients with a K_{ATP} channel mutation and those with a chromosome 6q24 anomaly. In addition to diabetes, seven patients with a 6q24 abnormality (30%) had macroglossia and two patients had umbilical hernia (9%). These abnormalities were not present in any of the patients with a K_{ATP} channel mutation. Patients with K_{ATP} channel TNDM have a higher birth weight (2,570 vs. 1,950 g), are diagnosed later (4 vs. 0 weeks), and enter remission later (35 vs. 13 weeks) than patients with 6q24 abnormalities. Although these clinical characteristics can aid in the identification of the cause of TNDM, there is overlap in the range of values (Table 2). Therefore, while clinical features can guide the order in which genetic tests are performed, a molecular genetic diagnosis is still required.

Phenotype of TNDM versus PNDM mutations. There is a clear genotype/phenotype relationship. None of the K_{ATP} channel mutations identified within this series have been found in patients with PNDM. Only one report, to date, has identified R201H, a well-characterized PNDM mutation, in a patient with TNDM (6). Between the TNDM and PNDM groups, there are no significant differences between the percentile birth weights and age of diagnosis (12th vs. 3rd percentile and 4 vs. 5 weeks) (16). Distinction between the two subgroups can be made only by the presence of a period of remission in the TNDM patients. One difference between the groups is the mode of inheritance. In our series, a greater number of probands (68%) have a familial mutation. This is in contrast to PNDM, in which 84% of all mutations occur de novo (21).

Therapeutic implications. Identification of a K_{ATP} channel mutation has important implications for patient clinical management. Recent reports (19,25,26) have shown that most patients with a *KCNJ11* mutation and PNDM are able to transfer from insulin onto sulfonylurea treatment with an improvement in glycemic control in all cases reported. Babenko et al. (8) reported two patients with an *ABCC8* mutation who were successfully treated with sulfonylureas after their TNDM relapsed. In our series, 3 of the 14 patients who developed diabetes outside the initial 6 months (either as a relapse or initial diagnosis) were initially treated with sulfonylureas. Since the diagnosis was made, a further five patients have successfully transferred onto sulfonylureas from insulin using a similar protocol to that described for the transfer of patients with PNDM and a *KCNJ11* mutation (18). For the remaining patients, transfer is either in progress or the patients are currently in remission.

In conclusion we have shown that a genetic diagnosis is possible for 97% of patients diagnosed with TNDM. Mutations of the K_{ATP} channel genes, *ABCC8* and *KCNJ11*, account for the majority (89%) of non-6q24 TNDM. Diabetes associated with these K_{ATP} channel mutations has a biphasic course, although neonatal diabetes that enters remission may not be detected. Patients therefore may present with permanent diabetes diagnosed in childhood or adulthood. In addition, not all mutation carriers may be diabetic when the mutation is detected in a proband. The therapeutic implications for those patients found to have a K_{ATP} channel mutation highlights the importance of screening all patients with non-6q24 TNDM for mutations in *ABCC8* and *KCNJ11*.

ACKNOWLEDGMENTS

We acknowledge funding from the Sir Graham Wilkins studentship to S.E.F. The study was funded by the Wellcome Trust and was supported by the European Union (Integrated Project EURODIA LSHM-CT-2006-518153 in the Framework Programme 6 of the European Community). A.T.H. is a Wellcome Trust Research Leave fellow. A.L.G. is a Diabetes UK R.D. Lawrence Research Fellow.

The authors thank all of the families for participating in

this study. Our thanks go to the many referring clinicians who include Ijaz Ahmad, Jesús Argente, Chandar Batra, Mark Bone, Jill Challener, Ethel Codner, Elizabeth Davis, Dorothy Deiss, Jan Fairchild, Peter Fowlie, Mathias Herr, Sabine Hofer, Mary Jetha, Olga Kordonouri, Michael MacDonald, Kathryn Noyes, Laurent Legault, Derek Sandeman, Annabelle Slingerland, Zdenek Sumnick, Peter Swift, Charalambos Theodoridis, Verena Wagner, and Esko Wiltshire. We also thank Andrew Parrish for his technical assistance and Dr. Beverley Shields for statistical analysis.

REFERENCES

1. Temple IK, Gardner RJ, Mackay DJ, Barber JC, Robinson DO, Shield JP: Transient neonatal diabetes: widening the understanding of the etiopathogenesis of diabetes. *Diabetes* 49:1359–1366, 2000
2. Temple IK, Gardner RJ, Robinson DO, Kibirige MS, Ferguson AW, Baum JD, Barber JCK, James RS, Shield JPH: Further evidence for an imprinted gene for neonatal diabetes localised to chromosome 6q22–q23. *Hum Molec Genet* 5:1117–1124, 1996
3. Gardner RJ, Mackay DJ, Mungall AJ, Polychronakos C, Siebert R, Shield JP, Temple IK, Robinson DO: An imprinted locus associated with transient neonatal diabetes mellitus. *Hum Mol Genet* 9:589–596, 2000
4. Gloyn AL, Reimann F, Girard C, Edghill EL, Proks P, Pearson ER, Temple IK, Mackay DJ, Shield JP, Freedenberg D, Noyes K, Ellard S, Ashcroft FM, Gribble FM, Hattersley AT: Relapsing diabetes can result from moderately activating mutations in KCNJ11. *Hum Mol Genet* 14:925–934, 2005
5. Yorifuji T, Nagashima K, Kurokawa K, Kawai M, Oishi M, Akazawa Y, Hosokawa M, Yamada Y, Inagaki N, Nakahata T: The C42R mutation in the Kir6.2 (KCNJ11) gene as a cause of transient neonatal diabetes, childhood diabetes, or later-onset, apparently type 2 diabetes mellitus. *J Clin Endocrinol Metab* 90:3174–3178, 2005
6. Colombo C, Delvecchio M, Zecchino C, Faienza MF, Cavallo L, Barbetti F: Transient neonatal diabetes mellitus is associated with a recurrent (R201H) KCNJ11 (KIR6.2) mutation. *Diabetologia* 48:2439–2441, 2005
7. Proks P, Arnold AL, Bruining J, Girard C, Flanagan SE, Larkin B, Colclough K, Hattersley AT, Ashcroft FM, Ellard S: A heterozygous activating mutation in the sulphonylurea receptor SUR1 (ABCC8) causes neonatal diabetes. *Hum Mol Genet* 15:1793–1800, 2006
8. Babenko AP, Polak M, Cave H, Busiah K, Czernichow P, Scharfmann R, Bryan J, Aguilar-Bryan L, Vaxillaire M, Froguel P: Activating mutations in the ABCC8 gene in neonatal diabetes mellitus. *N Engl J Med* 355:456–466, 2006
9. Temple IK, Shield JP: Transient neonatal diabetes, a disorder of imprinting. *J Med Genet* 39:872–875, 2002
10. Edghill EL, Dix RJ, Flanagan SE, Bingley PJ, Hattersley AT, Ellard S, Gillespie KM: HLA genotyping supports a nonautoimmune etiology in patients diagnosed with diabetes under the age of 6 months. *Diabetes* 55:1895–1898, 2006
11. Iafusco D, Stazi MA, Cotichini R, Cotellessa M, Martinucci ME, Mazzella M, Cherubini V, Barbetti F, Martinetti M, Cerutti F, Prisco F: Permanent diabetes mellitus in the first year of life. *Diabetologia* 45:798–804, 2002
12. Edghill EL, Gloyn AL, Goriely A, Harries LW, Flanagan SE, Rankin J, Hattersley AT, Ellard S: Origin of de novo KCNJ11 mutations and risk of neonatal diabetes for subsequent siblings. *J Clin Endocrinol Metab* 92:1773–1777, 2007
13. Mackay DJ, Temple IK, Shield JP, Robinson DO: Bisulphite sequencing of the transient neonatal diabetes mellitus DMR facilitates a novel diagnostic test but reveals no methylation anomalies in patients of unknown aetiology. *Hum Genet* 116:255–261, 2005
14. Mackay DJ, Boonen SE, Clayton-Smith J, Goodship J, Hahnemann JM, Kant SG, Njolstad PR, Robin NH, Robinson DO, Siebert R, Shield JP, White HE, Temple IK: A maternal hypomethylation syndrome presenting as transient neonatal diabetes mellitus. *Hum Genet* 120:262–269, 2006
15. Temple IK, James RS, Crolla JA, Sitch FL, Jacobs PA, Howell WM, Betts P, Baum JD, Shield J: An imprinted gene(s) for diabetes? *Nat Genet* 9:110–112, 1995
16. Flanagan SE, Edghill EL, Gloyn AL, Ellard S, Hattersley AT: Mutations in KCNJ11, which encodes Kir6.2, are a common cause of diabetes diagnosed in the first 6 months of life, with the phenotype determined by genotype. *Diabetologia* 49:1190–1197, 2006
17. Hattersley AT, Ashcroft FM: Activating mutations in kir6.2 and neonatal diabetes: new clinical syndromes, new scientific insights, and new therapy. *Diabetes* 54:2503–2513, 2005
18. Pearson ER, Flechtner I, Njolstad PR, Malecki MT, Flanagan SE, Larkin B, Ashcroft FM, Klimes I, Codner E, Iotova V, Slingerland AS, Shield J, Robert JJ, Holst JJ, Clark PM, Ellard S, Sovik O, Polak M, Hattersley AT: Switching from insulin to oral sulfonylureas in patients with diabetes due to Kir6.2 mutations. *N Engl J Med* 355:467–477, 2006
19. Girard CA, Shimomura K, Proks P, Absalom N, Castano L, Perez de Nanclares G, Ashcroft FM: Functional analysis of six Kir6.2 (KCNJ11) mutations causing neonatal diabetes. *Pflugers Arch* 453:323–332, 2006
20. Kondrashov AS: Direct estimates of human per nucleotide mutation rates at 20 loci causing mendelian disease. *Hum Mutat* 21:21–27, 2002
21. Slingerland AS, Hattersley AT: Mutations in the Kir6.2 subunit of the KATP channel and permanent neonatal diabetes: new insights and new treatment. *Ann Med* 37:186–195, 2005
22. Owen KR, Stride A, Ellard S, Hattersley AT: Etiological investigation of diabetes in young adults presenting with apparent type 2 diabetes. *Diabetes Care* 26:2088–2093, 2003
23. Shields BM, Knight B, Shakespeare L, Babrah J, Powell RJ, Clark PM, Hattersley AT: Determinants of insulin concentrations in healthy 1-week-old babies in the community: applications of a bloodspot assay. *Early Hum Dev* 82:143–148, 2006
24. Ma D, Shield JP, Dean W, Leclerc I, Knauf C, Burcelin RR, Rutter GA, Kelsey G: Impaired glucose homeostasis in transgenic mice expressing the human transient neonatal diabetes mellitus locus, TNDM. *J Clin Invest* 114:339–348, 2004
25. Zung A, Glaser B, Nimri R, Zadik Z: Glibenclamide treatment in permanent neonatal diabetes mellitus due to an activating mutation in Kir6.2. *J Clin Endocrinol Metab* 89:5504–5507, 2004
26. Tonini G, Bizzarri C, Bonfanti R, Vanelli M, Cerutti F, Faleschini E, Meschi F, Prisco F, Ciacco E, Cappa M, Torelli C, Cauvin V, Tumini S, Iafusco D, Barbetti F: Sulfonylurea treatment outweighs insulin therapy in short-term metabolic control of patients with permanent neonatal diabetes mellitus due to activating mutations of the KCNJ11 (KIR6.2) gene. *Diabetologia* 49:2210–2213, 2006
27. Cole TJ, Freeman JV, Preece MA: British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. *Stat Med* 17:407–429, 1998