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Mutations in the *ABCC8* gene encoding the SUR1 subunit of the K_{ATP} channel cause transient neonatal diabetes, permanent neonatal diabetes or permanent diabetes diagnosed outside the neonatal period

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Abstract

Aim—Mutations in the *ABCC8* gene encoding the SUR1 subunit of the pancreatic K_{ATP} channel cause permanent (PNDM) and transient neonatal diabetes mellitus (TNDM). We reviewed the existing literature, extended the number of cases and explored genotype-phenotype correlations.

Methods—Mutations were identified by sequencing in patients diagnosed with diabetes before 6 months without a *KCNJ11* mutation.

Results—We identified *ABCC8* mutations in an additional 9 probands (including 5 novel mutations L135P, R306H, R1314H, L438F and M1290V), bringing the total of reported families to 48. Both dominant and recessive mutations were observed with recessive inheritance more common in PNDM than TNDM (9 vs 1; $p < 0.01$). The remainder of the PNDM probands (n=12) had *de novo* mutations. 17/25 children with TNDM inherited their heterozygous mutation from a parent. Nine of these parents had permanent diabetes (median age at diagnosis 27.5 years, range 13 - 35). Recurrent mutations of residues R1183 and R1380 were found only in TNDM probands and dominant mutations causing PNDM clustered within exons 2-5.

Conclusions— *ABCC8* mutations cause PNDM, TNDM or permanent diabetes diagnosed outside the neonatal period. There is some evidence that the location of the mutation is correlated with the clinical phenotype.

Keywords

ABCC8; sulfonylurea receptor-1; SUR1; Permanent Neonatal Diabetes; Transient Neonatal Diabetes; sulphonylureas; ATP-sensitive potassium ion channel

Introduction

Diabetes diagnosed within the first six months of life is likely to have a genetic aetiology [1, 2]. Transient neonatal diabetes mellitus (TNDM) is usually diagnosed in the first week, remits by a median age of 12 weeks and relapses in adolescence or early adulthood in up to half of patients [3]. In almost 70% of cases TNDM is associated with an abnormality

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of the imprinted region 6q24 [3]. Heterozygous gain-of-function mutations in *KCNJ11*, encoding the Kir6.2 subunit of the ATP-sensitive potassium channel (K_{ATP}), are found in some of the remaining cases [4–6]. Permanent neonatal diabetes mellitus (PNDM) has, until recently, required insulin treatment for life. Heterozygous activating mutations in *KCNJ11* are the most common cause, accounting for 30-50% of cases [5, 7–10]. Most patients with activating Kir6.2 mutations respond to treatment with high dose sulphonylureas [11]. Transfer from insulin to sulphonylurea tablets for these patients, not only improves quality of life but improves glycaemic control [11, 12].

Heterozygous activating mutations in the *ABCC8* gene encoding SUR1, the regulatory subunit of the K_{ATP} channel protein, have recently been identified as a cause of PNDM in infants diagnosed under six months of age [13–17] and also TNDM [13, 18]. It is clear from this body of work that mutations in the *ABCC8* gene are a common cause of both PNDM and TNDM. In addition, some family members with an activating *ABCC8* mutation have permanent diabetes diagnosed at a range of ages that may present as type 2 diabetes. This article aims to bring together the recent genetic and clinical work on SUR1 diabetes.

Role of the K_{ATP} channel in insulin secretion

K_{ATP} channels regulate electrical activity of the plasma membrane in many tissues and cell types including pancreatic beta-cells, heart, brain, kidney, skeletal and smooth muscle [reviewed in 19]. In these tissues membrane depolarisation is linked to stimulatory signals through control of potassium ion flux. Different isoforms of the pore forming and regulatory subunits are found particular to the cell type. The K_{ATP} channel of the pancreatic beta cell links the sensing of glucose metabolism with membrane electrical activity in order to stimulate insulin secretion, a link that was established more than 20 years ago [20].

The channel is comprised of four inwardly rectifying potassium ion pore-forming subunits (Kir6.2) and four high affinity sulphonylurea receptor subunits (SUR1) in an intra-membrane hetero-octomeric complex [21, 22]. The circulating blood glucose level is detected by the K_{ATP} channel through changes in the balance of cytosolic nucleotides (ATP and Mg-ADP) within the beta cell. Inhibition and therefore closure of the channel is associated with an increase in glucose levels and is induced by ATP binding to the Kir6.2 subunit [23]. This leads to beta cell membrane depolarisation and opening of the voltage-gated calcium ion channels. The subsequent calcium ion influx initiates beta-cell electrical activity and triggers a cascade of events that result in the secretion of insulin [24].

The SUR1 subunit provides a regulatory control for the K_{ATP} channel. Magnesium nucleotides that bind to SUR1 subunits activate the channel thus increasing the probability of an open channel conformation [23, 25]. Channel activity is dependent upon the ratio of adenine nucleotides ATP and Mg-ATP (as well as their hydrolysed derivatives) binding to the channel subunits with their antagonistic functions. The regulatory action of SUR1 on the open state of the channel acts through the cooperative nature of nucleotide binding at the domains NBD1 and NBD2 of ATP and Mg-ADP (or Mg-ATP with subsequent hydrolysis) respectively [26]. Sulphonylurea drugs interrupt this cooperative binding by removing the bound Mg-ADP at NBD2, destabilising any bound ATP at NBD1 and facilitating channel closure [26]. The ability of sulphonylureas to bypass metabolic stimulation and close K

$_{ATP}$ channels, hence allowing the patients' own endogenous insulin to be released, has been widely exploited in the treatment of type 2 diabetes [27].

ABCC8 as a candidate gene for neonatal diabetes

The crucial role of the K_{ATP} channel in insulin secretion and the regulatory function of SUR1 suggested that *ABCC8* gene was a good candidate in which to identify mutations that could lead to the potential disruption of glucose homeostasis. The human *ABCC8* gene that encodes the SUR1 protein is located on chromosome 11p15.1 and consists of 39 exons (accession number NM_000352.2). It spans over 100Kb of genomic DNA and is adjacent to the *KCNJ11* gene encoding Kir6.2. The SUR1 protein is predicted, through membrane topology studies, to contain three transmembrane domains (TMD0, TMD1 and TMD2) linked by the cytosolic linker region (L0) and two nucleotide binding domains (NBD1 and NBD2). Two such domains each contain six transmembrane helices and the third, sited at the hydrophobic N-terminus and important for interactions with Kir6.2, contains five transmembrane helices [28]. Each of the nucleotide binding domains contain sequence motifs called Walker A and Walker B which are essential for binding the phosphate groups of nucleotides [29].

Hyperinsulinemia is characterised by oversecretion of insulin despite hypoglycaemia. Recessively inherited loss-of-function mutations in the *ABCC8* gene are the most common cause of congenital hyperinsulinemic hypoglycaemia [30, 31]. Inactivating mutations in *KCNJ11* also cause HI although they are rarer than *ABCC8* mutations [32–34]. Transgenic mice that overexpress Kir6.2 developed the opposite phenotype of severe hyperglycaemia, hypoinsulinemia and ketoacidosis within 2 days [35]. Activating mutations in *KCNJ11* are now known to cause both PNDM and TNDM in humans [6, 7]. *ABCC8* was therefore an excellent candidate gene for neonatal diabetes and during the past year several reports have described activating mutations in the *ABCC8* gene in patients with PNDM or TNDM [13–18].

Methods

Patients with diabetes diagnosed in the first six months of life, in which 6q24 abnormalities and *KCNJ11* mutations had been excluded were recruited. Requests for referrals were made to the International Society of Paediatric and Adolescent Diabetes (ISPAD) rare diabetes collection. The study was conducted in accordance with the Declaration of Helsinki as revised in 2000. Informed consent was obtained from all participating patients with parental consent given on behalf of children.

Genetic Analysis

Genomic DNA was extracted from peripheral lymphocytes using standard procedures. In patients with a TNDM phenotype analysis of the 6q24 locus was undertaken using previously described methods to detect duplications, uniparental disomy (UPD) and methylation abnormalities [3, 36]. In patients where no 6q24 abnormality was identified or those with permanent diabetes, the single exon of *KCNJ11* was amplified in three overlapping fragments as previously described [5]. The 39 exons of *ABCC8* were analysed

in all patients where no *KCNJ11* mutation was identified. The *ABCC8* gene was amplified in 38 fragments using previously described primers [14]. PCR products were sequenced using standard methods on an ABI 3100 or ABI 3730 (Applied Biosystems, Warrington, UK). Sequences were compared to the published sequence (NM_000352.2 including the alternatively spliced residue in exon 17; L78208, L78224) using Mutation Surveyor v2.61 (Softgenetics, Pennsylvania, USA). Mutations were tested for co-segregation with diabetes in other family members and in 200 normal chromosomes. Where possible, family relationships were confirmed using a panel of six microsatellite markers on chromosome 11p [5].

Clinical characteristics were obtained from the patient's hospital records with assistance from their physician. Results are presented as median (range) and comparative statistics used the Mann-Whitney U test and Chi square statistic with Yates correction. Birth weight centiles were calculated using the method described by Cole [37].

Results

ABCC8 mutations causing diabetes mellitus

ABCC8 mutations have been reported previously in 39 index cases with neonatal diabetes [13–18]. In this article we describe a further 9 families (see Figure 1) bringing the total number to 48 (see Table1). These include 21 probands with PNDM, 25 with TNDM and two patients who have not had a period of remission without treatment but are receiving reduced doses of insulin (see Table 1).

Inheritance of mutations

The modes of inheritance of mutations in probands with PNDM and TNDM are illustrated in Figure 2. Dominantly acting heterozygous mutations account for 79% (38/48) of the index cases. Recessive inheritance was more common in PNDM than in TNDM cases (9 vs. 1; Chi square $p < 0.01$). Spontaneous mutations accounted for 57% (12/21) of PNDM and 32% (8/25) of TNDM probands (family relationships were confirmed where possible by microsatellite marker analysis). The remaining 16 TNDM probands showed dominant inheritance of a heterozygous mutation from a parent. In contrast, no families have been described with dominantly inherited PNDM due to an *ABCC8* mutation.

Characteristics of mutations

A total of 39 different missense and 2 frameshift *ABCC8* mutations have been identified in patients with diabetes. None of these mutations have been reported as loss-of-function mutations causing hyperinsulinism. The majority of mutations result in missense amino acid substitutions distributed across the gene (Figure 3). Nearly half (18) of these missense mutations are located in exons 2-6. A particular mutation hotspot is exon 5 where 10 different mutations have been identified. These mutations affect residues 207 to 263 which lie within the cytosolic linker (L0) between transmembrane domains TMD0 and TMD1 (Figure 4).

Five novel mutations were identified; L135P (c.404T>C), R306H (c.917G>A), R1314H (c.3941G>A), L438F (c.1312C>T) and M1290V (c.3868A>G). The substituted amino acids are conserved across vertebrates including human, mouse, rat and dog and were not observed in at least 200 normal chromosomes.

Five residues are sites for different amino acid substitutions; V86A/G, F132L/V, D212I/N, R1183Q/W and R1380C/H/L. Nine mutations were observed in more than one proband; R1183W (c.3547C>T) was identified in five probands, R1380C (c.4138C>T) in three probands and the remainder; F132L (c.394T>C), D209E (c.627C>A), T229I (c.686C>T), L582V (c.1744C>G), R826W (c.2476C>T), R1183Q (c.3548G>A) and R1380L (c.4139G>T) were each observed in two probands. The recurrent mutations at codons R1183 and R1380 result in a base substitution within a CpG dinucleotide (c.4138-4139 or c.3547-3548). CpG dinucleotides are well known sites prone to mutation through the deamination of methylated cytosine bases.

Mutations in probands with PNDM

PNDM was caused either by a *de novo* dominantly acting heterozygous mutation (n=12) or recessive inheritance of mutations from unaffected parents with heterozygous mutations (n=9). We report two new families with PNDM; a novel, *de novo* L135P mutation (ISPAD146) and one with recessive inheritance (ISPAD142). Most (9/10) dominantly acting missense mutations in patients with PNDM were found in exons 2, 3 and 5 (Figure 3) which encode the TMD0 and cytosolic linker L0 regions of SUR1. Recessively inherited missense mutations were more widely distributed throughout exons 1-8 and 28-38. Mutations affecting residues V86 (n=2) or F132 (n=3) were found only in probands with PNDM.

Eight probands inherited a heterozygous mutation from each parent. Two are the offspring of consanguineous relationships (parents are first cousins) and are homozygous for a missense mutation (ISPAD117 and118, [14]. Compound heterozygous mutations that cause neonatal diabetes were first described in 5 families by Ellard *et al.* [14] and a further family is reported here (two affected siblings who are compound heterozygotes for the novel L438F and M1290V mutations; ISPAD142). Compound heterozygous mutations may include two activating mutations or an activating mutation inherited *in trans* with a loss-of-function mutation [14]. A case of mosaic uniparental disomy in combination with an *ABCC8* mutation has also been described in a child with PNDM whose unaffected father was heterozygous for the mutation [14].

Mutations in probands with TNDM and their relatives

We report 5 new families where the proband had transient neonatal diabetes (see Figure 1). They include the novel missense mutation R1315H (ISPAD144), three families with the previously reported mutations R826W, R1183W and R1380L [18] and the first case of a homozygous mutation (T229I) causing TNDM (ISPAD62).

A total of 17 different mutations have now been reported in TNDM and these are located in exons 5-34 (Figure 3). Mutations affecting residues D212 (n=2), L582 (n=2), R826 (n=2), R1183 (n=7) or R1380 (n=6) were found only in probands with TNDM.

The majority of TNDM probands (16/25) inherited a heterozygous mutation from a parent. Nine of the mutation carrier parents had permanent diabetes (median age at diagnosis 27.5 years, range 13 - 35), but only two of these had been diagnosed with hyperglycaemia during the neonatal period. Two probands each had a sibling affected with TNDM [18] and in three families (ISPAD126; [13] there was a history of adult-onset diabetes affecting a grandparent (*ABCC8* mutation confirmed in two).

Clinical phenotype

Table 2 shows a comparison of the clinical data for all mutation carriers. Affected probands and family members can be separated into three distinct groups based on age of diagnosis and whether there was a period of remission (or reduced insulin dose). The calculated median birth weight for the PNDM and TNDM cases were the12th and 16th centiles respectively (p=0.69). TNDM cases were diagnosed earlier than PNDM at a median of 3 vs 7 weeks of age (p<0.01). The median age of diagnosis for all cases of diabetes that were diagnosed after 6 months is 30 years of age (range 13-52) and the current median age of the unaffected mutation carrier parents is 36 years (range 25-42).

Response to sulphonylurea therapy

Eleven patients have previously been reported to have successfully transferred from insulin to sulphonylurea tablets [13, 15, 17, 18]. The patient with DEND syndrome and the F132L mutation was not able to discontinue insulin [16]. An additional 7 patients have now stopped insulin as a result of their molecular genetic diagnosis (Table 1).

Neurological features

Neurological features were reported in 15/48 (31%) probands with 13 different mutations (see Table 1 for details). One patient (ISPAD68) has previously been reported with DEND syndrome [16]. A second patient with the same mutation (F132L) had developmental delay but no epilepsy. No neurological features were reported in a third patient with a different mutation at the same residue (F132V).

Developmental delay was seen in both probands with the R1380L mutation. Further complications suggestive of DEND syndrome were also observed in these patients including abnormalities on electro encephalogram (EEG) in one patient and reported seizures in the second. One of the three patients with the R1380C mutation was reported to have minor dystonia [13]. No neurological features were reported in the patient with the R1380H mutation [18].

One of the six TNDM probands with the R1183W mutation experienced neurological problems prior to starting insulin treatment. He had one episode of tonic posturing with right facial involvement following admission to hospital with diabetic ketoacidosis. He subsequently had two episodes of generalised seizures but no further episodes since insulin therapy was started [18].

Muscle weakness was identified in two cousins with the D212I mutation. The proband had muscle hypotonia until the age of 8 months and her cousin has also been diagnosed with

motor developmental delay. No muscle weakness was reported in either of their affected mothers or the three carriers of the D212N mutation [18].

Of the 9 PNDM probands with recessive mutations, two had neurological features (muscle weakness and seizures or learning difficulties) which were not found in their affected siblings. Neurological features were identified in 4/12 probands with dominant mutations.

Genotype-phenotype correlation

Most of the dominantly acting mutations located in exons 2-5 of the *ABCC8* gene (V86A/G, F132L/V, L135P, D209E, Q211K, L213R and L225P) cause PNDM. The exceptions are D212I and D212N that result in a remitting/relapsing phenotype with permanent diabetes in later life. Two arginine residues in exons 28 and 34 are hotspots for recurrent mutations (R1183Q/W and R1380C/H/L) found only in patients with TNDM or adult-onset permanent diabetes (n=18).

There are examples of phenotypic variability both between and within families. The heterozygous mutation D209E has been reported as *de novo* in one proband (current age 6 yrs) with PNDM [14] and in a second family where the proband had TNDM but her mother was diagnosed with diabetes at 35 years of age [18]. New data from the previously reported family ISPAD47 [14] reveals that the proband (now aged 8 years) has permanent diabetes but his younger sister (now aged 7 years) had transient diabetes that remitted at the age of 2 years. Both are compound heterozygotes for the P45L and G1401R mutations.

Discussion

We report *ABCC8* mutations in a further 9 probands and present a summary that includes the 39 previously reported families. A spectrum of phenotypes is observed in patients with diabetes caused by *ABCC8* mutations as has previously been reported for *KCNJ11* mutations [38]. These include DEND syndrome [16], permanent diabetes diagnosed before 6 months of age [13–15, 17], transient neonatal diabetes [13, 18] or permanent diabetes diagnosed in adolescence or adulthood [13, 18]. The latter two phenotypes are usually found within the same family where a proband was referred for testing because of a diagnosis of transient neonatal diabetes and an affected parent (and/or grandparent) may have permanent diabetes. This implies that these K_{ATP} channel mutations have a biphasic course and patients diagnosed later may have had a period of hyperglycaemia that was undetected in the neonatal period [18].

Recessive *ABCC8* mutations were first reported as a cause of diabetes by Ellard *et al.* [14]. Although there are no reports of recessive *KCNJ11* mutations, such mutations are not uncommon in *ABCC8*, accounting for 19% of all probands. All except one has PNDM. *De novo ABCC8* mutations explain 42% of neonatal diabetes cases but are more common in PNDM (57%) than TNDM (32%). This pattern is also seen for *de novo KCNJ11* mutations; 84% in PNDM [39] vs 29% in TNDM (Flanagan 2007). The remainder are dominantly inherited heterozygous mutations found exclusively in patients with TNDM or permanent diabetes diagnosed outside the neonatal period. The mode of inheritance is important since it determines the risk of diabetes in future siblings, offspring, parents and the wider family.

The clinical phenotype for TNDM vs PNDM mutations is similar as measured by birth weight (a surrogate for insulin secretion *in utero*) which was reduced in both groups (16^{th} vs 12^{th} centiles respectively; p=0.69). Probands with TNDM were diagnosed earlier 3 vs 7 weeks of age (p<0.01) and neurological features were present in a minority (31%) of index cases.

Many patients with SUR1 subunit mutations can transfer from insulin to sulphonylurea treatment and at least 18 patients are known to have achieved successful transfer. There are no reports of patients with DEND syndrome caused by either *ABCC8* or *KCNJ11* mutations who have been able to stop insulin treatment. No data is yet available on the level of glycaemic control on sulphonylureas compared to insulin for patients with SUR1 mutations. A number of *ABCC8* mutation carriers who are not known to have diabetes have been identified [13, 18, this study] but not all have undergone a formal OGTT. Annual blood glucose monitoring is recommended for this group who may benefit from sulphonylurea treatment if/when they develop diabetes.

A genotype-phenotype relationship for SUR1 mutations is suggested by the clustering of dominantly acting mutations in exons 2-5 that cause PNDM and the recurrent mutations of the arginine residues at codons 1183 and 1380 that only cause TNDM or permanent diabetes diagnosed outside the neonatal period. Furthermore, different mutations at the same residue (V86A/G, F132L/V, D212I/N, R1183Q/W and R1380C/H/L) cause either PNDM (V86, F132) or biphasic TNDM (D212, R1183, R1380), suggesting a different pathological mechanism.

The N-terminal transmembrane domain (TMD0) and cytosolic linker (L0) form allosteric interactions with Kir6.2 that are essential for normal intrinsic activity and increase channel density in the membrane [25]. Residues in the L0 region confer constitutive channel activity and together with TMD0 have been implicated in the intrinsic gating properties of the K_{ATP} channel [40, 41]. The cluster of NDM causing mutations in the first five exons of the *ABCC8* gene that encode these regions might cause diabetes by increasing the open stability of the channel through interaction with the Kir6.2 subunit as demonstrated for the F132L mutation [16]. The phenotypic differences between different mutations in this region might provide evidence to suggest which residues interact most closely with the Kir6.2 subunits. The dominant heterozygous mutations causing a PNDM phenotype could therefore highlight residues that directly influence gating whilst the recessively inherited or TNDM causing mutations in the same region may identify residues with less influence.

The most common mutations in patients with TNDM/permanent diabetes diagnosed in adolescence or adulthood affect the arginine residues R1183 and R1380. These residues are implicated in nucleotide binding, since R1183 is located at a position involved in the joining of transmembrane domain 2 (TMD2) to nucleotide binding domain 2 (NBD2) and R1380 is located within NBD2. NBD2 is important for the regulation of channel activity by preferentially binding magnesium nucleotides Mg-ATP or Mg-ADP [26, 41] and in the 3D structure R1380 lies adjacent to the ATP binding site of NBD1 [42]. Loss-of-function mutations in these regions of *ABCC8* can prevent binding of ATP and abolish responsiveness of SUR1 to MgADP leading to congenital hyperinsulinaemic hypoglycaemia

[43]. However activating mutations in these NBD regions have now been reported (e.g. R826W, R1380C/H/L, V1523A/L) that could act to enhance the stimulatory effect of magnesium nucleotide binding or reduce the hydrolysis rate of Mg-ATP [16].

Neurological features in addition to neonatal diabetes were identified in 31% of probands with an *ABCC8* mutation. They include developmental delay, muscle weakness and epilepsy, but were not strongly associated with mutation position in the gene or consistent between carriers of the same mutation. K_{ATP} channels are found in many other tissues but the expression pattern of *ABCC8* restricts the pairing of the SUR1 protein subunits with Kir6.2 to the beta cell and brain [19]. The neurological complications observed in probands with *ABCC8* mutations could result from disrupted neuronal signalling and/or glucose homeostasis. Seizures were observed in three of the probands although they may have occurred secondary to severe hyperglycaemia. In support of this, none of the patients with onset of diabetes outside the neonatal period exhibited neurological features.

There is some evidence that activating mutations causing recessively inherited neonatal diabetes may have a milder functional effect compared to dominant SUR1 or Kir6.2 mutations [14], but some overlap was observed between the functional effects of the mutations present in unaffected heterozygotes and those in patients with neonatal diabetes. We report the first patient with TNDM and a homozygous *ABCC8* mutation (T229I). This mutation has previously been found in a child with PNDM who is a compound heterozygote for T229I and V1523L. Functional studies of heterozygous mutant channels suggested that V1523L has a greater effect on ATP sensitivity than T229I [14] and is consistent with the more severe PNDM phenotype caused by the T229I/V1523L genotype.

Although it is not possible to predict with confidence the clinical phenotype from the position of an *ABCC8* mutation, we have shown that there are some emerging patterns of specific mutations associated with clinical subtypes. The phenotypic variability observed both between and within some families with the same *ABCC8* mutation means that genotype-phenotype correlations are not absolute. Functional data has only been published for 8/39 *ABCC8* missense mutations to date (F132L, [16]; I1425V and H1024Y, [13]; mutations (P207S, T229I, A1185E and V1523L, [14]; L225P, [15]). Further studies of additional mutations may help to explain the mechanisms underlying these apparent genotype-phenotype correlations and will further our understanding of beta-cell K_{ATP} channel biology. The discovery that *ABCC8* mutations account for a significant number of *KCNJ11*-negative cases of neonatal diabetes strengthens the view that interactions between Kir6.2 and SUR1 are crucial for correct channel function and thus the appropriate secretion of insulin.

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Abbreviations

DEND	Developmental delay, Epilepsy and Neonatal Diabetes
PNDM	Permanent Neonatal Diabetes
TNDM	Transient Neonatal Diabetes
ISPAD	International Society of Paediatric and Adolescent Diabetes
K_{ATP}	ATP-sensitive potassium channel
NBD	Nucleotide Binding Domain
L0	SUR1 cytosolic linker region

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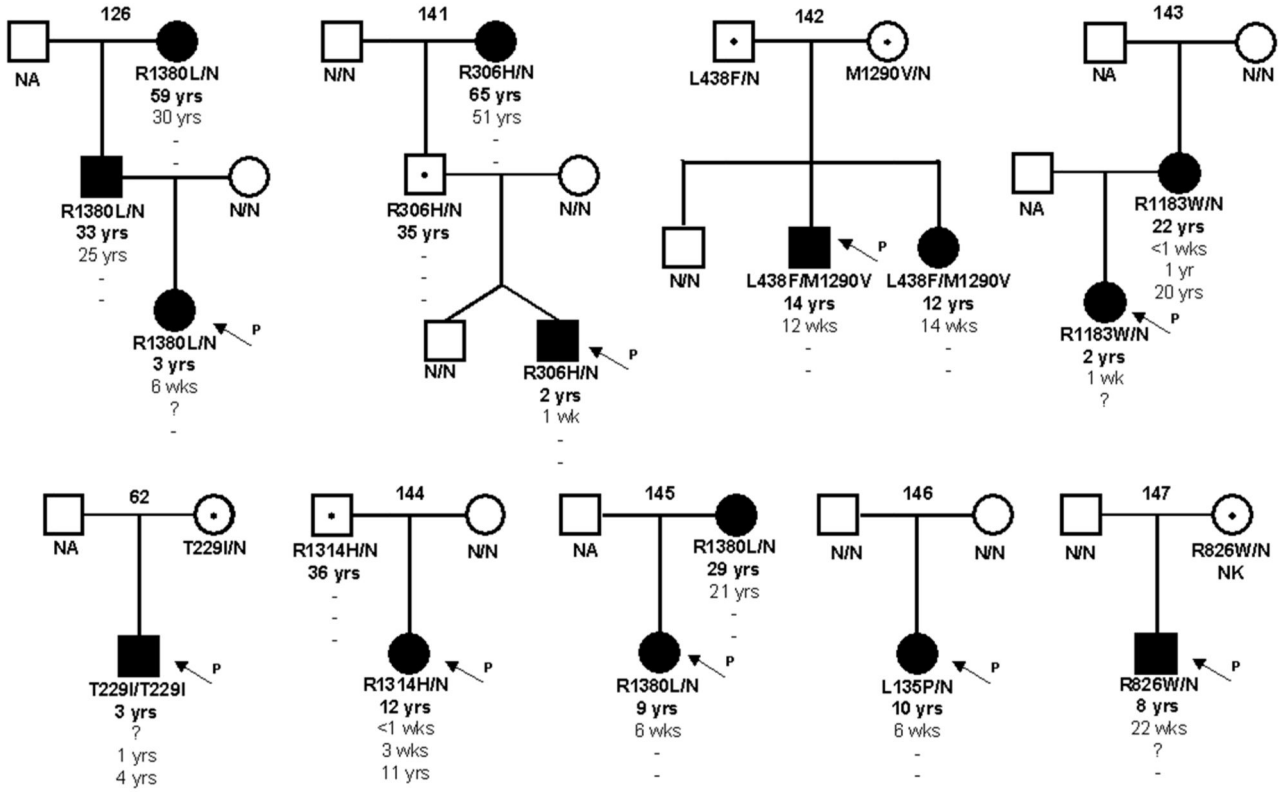

Figure 1.

Partial pedigrees for newly identified families showing inheritance of *ABCC8* mutations. Circles represent females and squares males. Filled symbols represent diabetes. Genotype is shown underneath each symbol with residue number and amino acid change for the mutation carriers, N/N denotes no mutation identified. For the mutation carriers their current age, the age of initial diagnosis, the age of remission and the age of relapse is given. A dash represents a non-event,? indicates age at time of diagnosis or remission is not known. NA denotes not available for genetic testing and NK signifies that clinical details were not known. An arrow points to the proband in each family.

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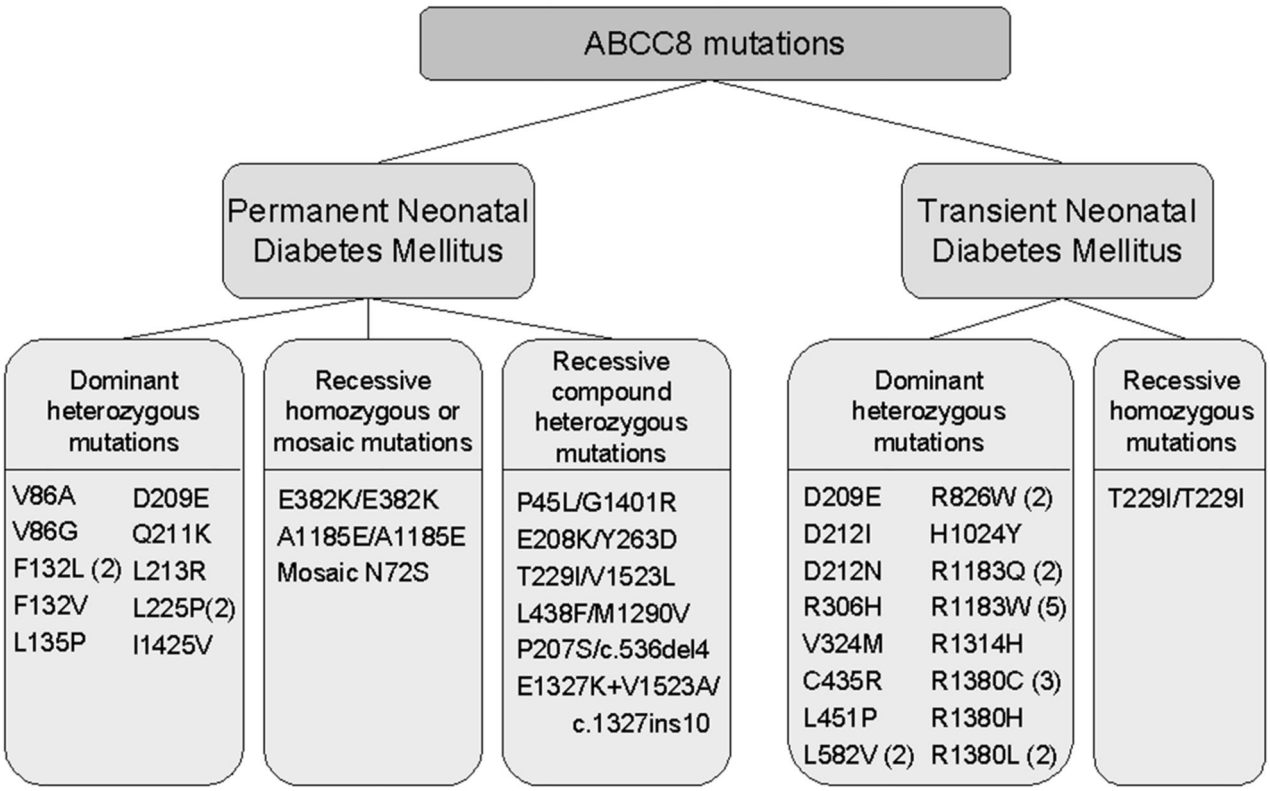

Figure 2.

A diagram illustrating the inheritance of *ABCC8* mutations in probands with permanent and transient forms of neonatal diabetes. A figure in brackets indicates the number of probands identified with that mutation where this is more than one.

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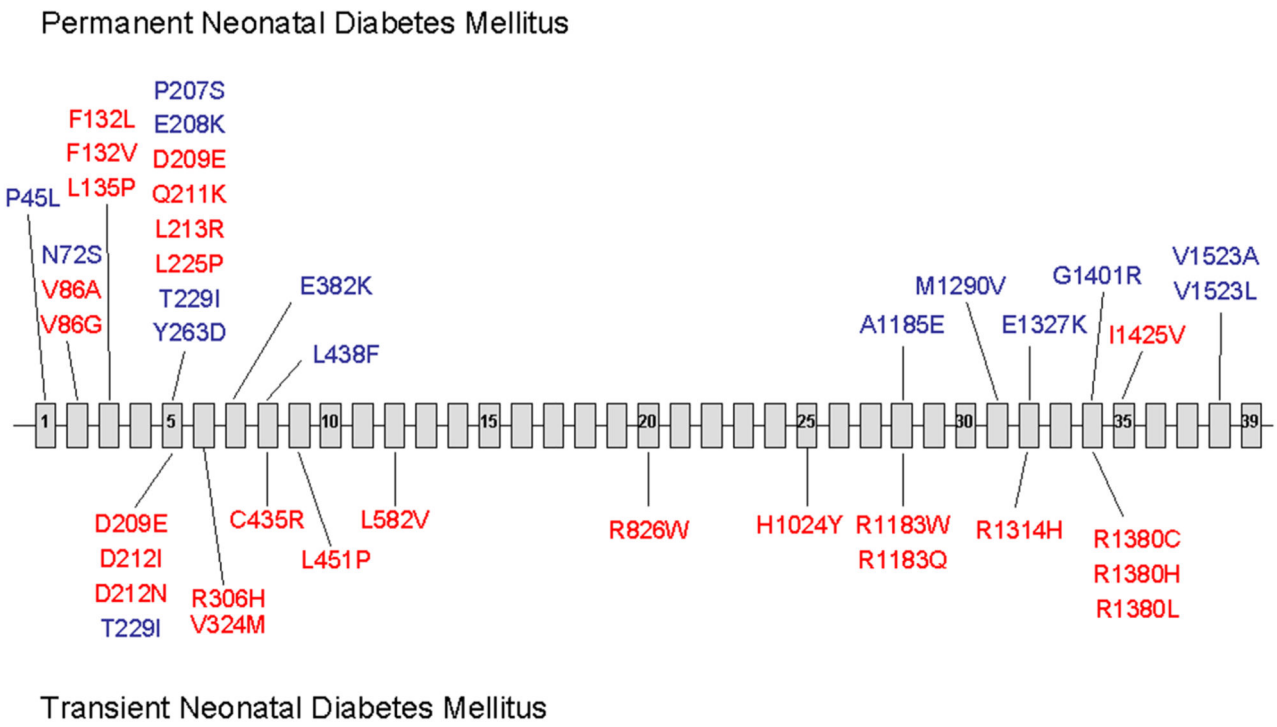

Figure 3.

The location of missense mutations causing neonatal diabetes within the coding sequence of *ABCC8*. The mutations above the line indicate those associated with permanent neonatal diabetes (PNDM) and those below are mutations associated with transient neonatal diabetes. Recessively inherited mutations are in blue with dominantly inherited mutations in red.

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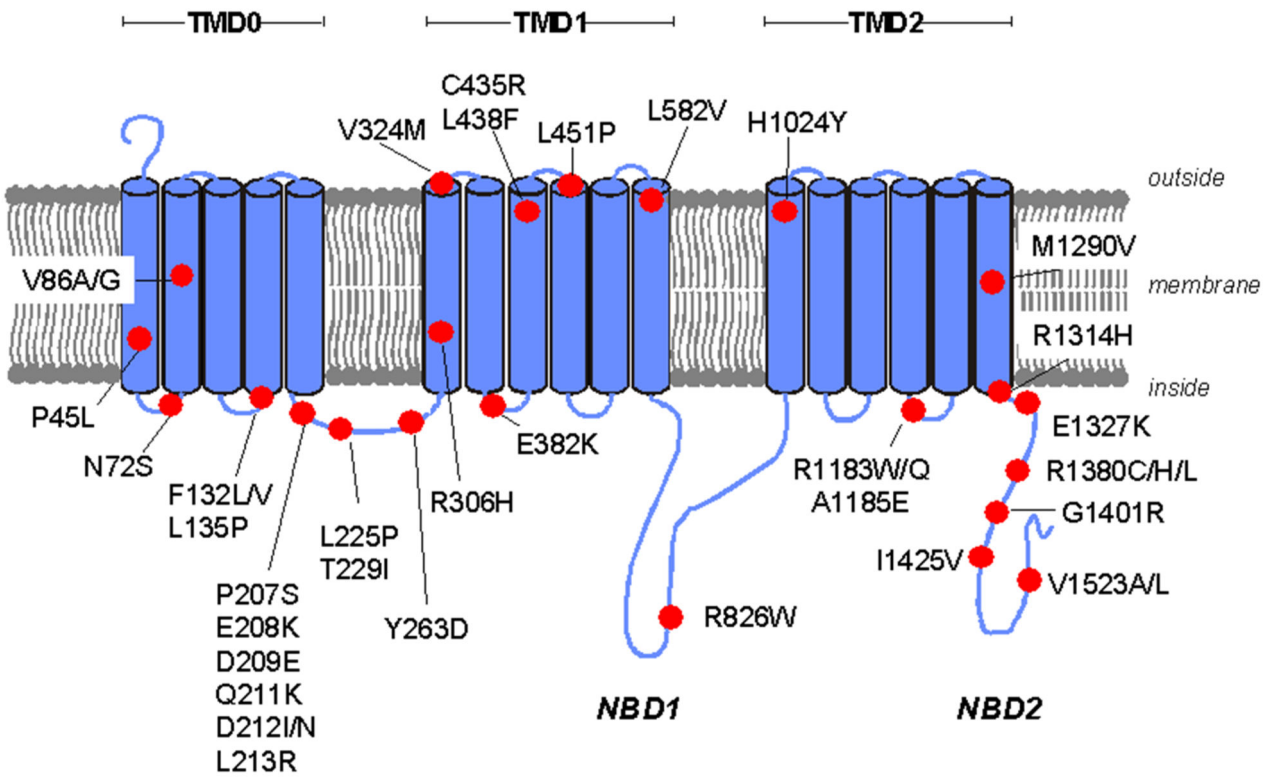

Figure 4.

A schematic of the membrane topologies of SUR1 showing the location of the *ABCC8* missense mutations causing neonatal diabetes. The transmembrane domains are indicated by TMD0, TMD1 and TMD2. The nucleotide binding domains are indicated by NBD1 and NBD2 and the cytosolic linker L0 is between TMD0 and TMD1.

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Table 1

***ABCC*8 mutations identified in probands with neonatal diabetes.**

ISPAD Number	Mutation (protein effect)	Nucleotide change	Zygosity	Phenotype	Country of origin	Age at Diagnosis (wks)	Birth weight g (Centile)	Neurological features	SU Transfer	Reference
123	V86A	c.257T>C	Heterozygous	PNDM	Slovakia	8	2900 (9)		Yes	Ellard et al. 2007; Stanik et al. 2006
124	V86G	c.257T>G	Heterozygous	PNDM	Canada	5	2900 (13)		Yes	Ellard et al. 2007
68	F132L	c.394T>C	Heterozygous	PNDM	Netherlands	13	2200 (<1)	Developmental and motor delay, hypotonia and epilepsy		Ellard et al. 2007; Proks et al. 2006
125	F132L	c.394T>C	Heterozygous	PNDM	Australia	26	2440 (9)	Developmental and motor delay		Ellard et al. 2007
82	F132V	c.394T>G	Heterozygous	PNDM	Poland	20	NA			Ellard et al. 2007
146	L135P	c.404T>C	Heterozygous	PNDM	Netherlands	6	N/N	Developmental and motor delay		
46	D209E	c.627C>A	Heterozygous	PNDM	USA	5	2720 (13)		Yes	Ellard et al. 2007
133	D209E	c.627C>A	Heterozygous	TNDM	Australia	3	2460 (9)		Yes	Flanagan et al. 2007
134	Q211K	c.631C>A	Heterozygous	PNDM	Chile	16	2400 (3)		Yes	Ellard et al. 2007
127	D212I	c.635A>T	Heterozygous	TNDM	Germany	4	2390 (16)	Muscle hypotonia up to age of 8 months		Flanagan et al. 2007
49	D212N	c.634G>A	Heterozygous	TNDM	Greece	4	2800 (12)			Flanagan et al. 2007
-	L213R	c.638T>G	Heterozygous	PNDM	N/A	17	3065 (22)	Developmental and motor delay, dyspraxia	Yes	Babenko et al. 2006
-	L225P	c.674T>C	Heterozygous	PNDM	US	6	2100(<1)		Yes	Masia et al. 2007
122	L225P	c.674T>C	Heterozygous	PNDM	Germany	4	2500 (11)			Ellard et al. 2007
141	R306H	c.917G>A	Heterozygous	*Not classified	UK	1	1290 (9)	Developmental delay		
24	V324M	c.970G>A	Heterozygous	TNDM	Spain	1	N/A			Flanagan et al. 2007
-	C435R	c.1303T>C	Heterozygous	TNDM	N/A	4	3040 (25)	Minor visual and spatial dyspraxia		Babenko et al. 2006
128	L451P	c.1352T>C	Heterozygous	TNDM	Austria	7	3030 (20)	Developmental delay		Flanagan et al. 2007
-	L582V	c. 1744C>G	Heterozygous	TNDM	N/A	10	3570 (67)			Babenko et al. 2006
-	L582V	c. 1744C>G	Heterozygous	TNDM	N/A	2	3350 (50)	Slow ideation		Babenko et al. 2006
8	R826W	c.2476C>T	Heterozygous	TNDM	UK	7	N/A			Flanagan et al. 2007
147	R826W	c.2476C>T	Heterozygous	TNDM	Poland	<1	N/A			

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ISPAD Number	Mutation (protein effect)	Nucleotide change	Zygosity	Phenotype	Country of origin	Age at Diagnosis (wks)	Birth weight g (Centile)	Neurological features	SU Transfer	Reference
-	H1024Y†	c.1370C>T	Heterozygous	TNDM	N/A	3	3400 (55)		Yes	Babenko et al. 2006
71	R1183Q	c.3548G>A	Heterozygous	TNDM	UK	1	1960 (<1)			Flanagan et al. 2007
-	R1183Q†	C.3548G>A	Heterozygous	TNDM	N/A	<1	1830 (8)			Babenko et al. 2006
143	R1183W	c.3547C>T	Heterozygous	TNDM	Germany	1	1340 (20)			
132	R1183W	c.3547C>T	Heterozygous	TNDM	UK	8	2600 (2)			Flanagan et al. 2007
131	R1183W	c.3547C>T	Heterozygous	TNDM	India	9	2800 (6)	Episodes of tonic posturing and seizures prior to insulin therapy		Flanagan et al. 2007
130	R1183W	c.3547C>T	Heterozygous	TNDM	Germany	6	2550 (4)			Flanagan et al. 2007
26	R1183W	c.3547C>T	Heterozygous	TNDM	Czech Republic	1	2400 (68)			Flanagan et al. 2007
144	R1314H	c.3941G>A	Heterozygous	TNDM	USA	<1	N/A		Yes	
72	R1380C	c.4138C>T	Heterozygous	TNDM	USA	8	3400 (23)			Flanagan et al. 2007
-	R1380C†	c.4138C>T	Heterozygous	TNDM	N/A	<1	2050 (<3)	Minor dystonia		Babenko et al. 2006
-	R1380C†	c.4138C>T	Heterozygous	TNDM	N/A	8	2330 (<3)		Yes	Babenko et al. 2006
65	R1380H	c.4139G>A	Heterozygous	TNDM	UK	1	1360 (32)			Flanagan et al. 2007
145	R1380L	c.4139G>T	Heterozygous	*Not classified	Romania	6	2700 (6)	Developmental delay with EEG abnormalities		
126	R1380L	c.4139G>T	Heterozygous	TNDM	Canada	6	2800 (4)	Developmental delay, seizures and dystonia		
-	I1425V†	c.4273A>G	Heterozygous	PNDM	N/A	4	3080 (25)		Yes	Babenko et al. 2006
62	T229I	c.686C>T	Homozygous	TNDM	UK	N/A	N/A			
117	E382K	c.1144G>A	Homozygous	PNDM	Turkey	8	2700 (4)			Ellard et al. 2007
118	A1185E	c.3554C>A	Homozygous	PNDM	Turkey	<1	4200 (95)	Muscle weakness and seizures		Ellard et al. 2007
116	N72S	c.215A>G	Mosaic	PNDM	UK	5	3870 (74)			Ellard et al. 2007
47	P45L + G1401R	[c.134C>T] + [c.4201G>A]	Compound heterozygous	PNDM	UK	6	2520 (18)		Yes	Ellard et al. 2007
119	E208K + Y263D	[c.622G>A] + [c.787T>G]	Compound heterozygous	PNDM	Canada	13	2950 (28)	Developmental delay	Yes	Ellard et al. 2007

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ISPAD Number	Mutation (protein effect)	Nucleotide change	Zygosity	Phenotype	Country of origin	Age at Diagnosis (wks)	Birth weight g (Centile)	Neurological features	SU Transfer	Reference
120	T229I + V1523L	[c.686C>T] + [c.4567G>T]	Compound heterozygous	PNDM	Canada	4	NA		Yes	Ellard et al. 2007
142	L438F + M1290V	c.1312C>T/c.3868A>G	Compound heterozygous	PNDM	Chile	12	3050 (43)			
78	P207S + c.536del4	[c.619C>T] + [c.536 539delATGG]	Compound heterozygous	PNDM	Germany	8	3290 (29)			Ellard et al. 2007
121	[E1327K; V1523A] +T1043QfsX74	[c.3979G>A; 4568C>T] + [c.3127_3129delACCinsCAGCCAGGACCTG]	Compound heterozygous	PNDM	USA	1	2380 (<1)			Ellard et al. 2007

† Amino acid numbering has been altered to include the alternatively spliced residue in exon 17 (L78208, L78224).

* ISPAD probands 141 and 145 were not classified as having either TNDM or PNDM since they have not stopped insulin treatment but are receiving reduced doses of insulin dose (current dose 0.4 and 0.18U/kg/day respectively).

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Table 2

Clinical characteristics of mutation carriers diagnosed with diabetes.

	PNDM† (n=24)	TNDM‡ (n=31)	DM∓ (n=11)
Sex (Number of Males)	12	16	4
Age at diagnosis	7 wks (<1-26)	3 wks (<1-22)	30 yrs(13-51)
Birth weight centile	12 (<1-95)	16 (<1-69)	-
- Any Neurological features	8	7	0
- Developmental delay	8	3	0
- Muscle Weakness	2	0	0
- Epilepsy	2	0	0

† Permanent Neonatal Diabetes with onset ≤ 6 months age and no remission

‡ Transient Neonatal Diabetes with onset ≤ 6 months age and a period of remission with no treatment

∓ Diabetes diagnosed >6 months of age.

Clinical characteristics are presented as median (range)