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ARTICLE

A Kir6.2 mutation causing severe functional effects in vitro produces neonatal diabetes without the expected neurological complications

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Abstract
Aims/hypothesis Heterozygous activating mutations in the pancreatic ATP-sensitive K^+ channel cause permanent neonatal diabetes mellitus (PNDM). This results from a decrease in the ability of ATP to close the channel, which thereby suppresses insulin secretion. PNDM mutations that cause a severe reduction in ATP inhibition may produce additional symptoms such as developmental delay and epilepsy. We identified a heterozygous mutation (L164P) in the pore-forming (Kir6.2) subunit of the channel in three unrelated patients and examined its functional effects.

Methods The patients (currently aged 2, 8 and 20 years) developed diabetes shortly after birth. The two younger patients attempted transfer to sulfonylurea therapy but were unsuccessful (up to 1.1 mg kg^{-1} day^{-1}). They remain insulin dependent. None of the patients displayed neurological symptoms. Functional properties of wild-type and mutant channels were examined by electrophysiology in *Xenopus* oocytes.

Results Heterozygous (het) and homozygous L164P K_{ATP} channels showed a marked reduction in channel inhibition by ATP. Consistent with its predicted location within the pore, L164P enhanced the channel open state, which explains the reduction in ATP sensitivity. HetL164P currents exhibited greatly increased whole-cell currents that were unaffected by sulfonylureas. This explains the inability of sulfonylureas to ameliorate the diabetes of affected patients.

Conclusions/interpretation Our results provide the first demonstration that mutations such as L164P, which produce a severe reduction in ATP sensitivity, do not inevitably cause developmental delay or neurological problems. However, the neonatal diabetes of these patients is unresponsive to sulfonylurea therapy. Functional analysis of PNDM mutations can predict the sulfonylurea response.

Keywords K_{ATP} channel · *KCNJ11* · Kir6.2 · Neonatal diabetes · Type 2 diabetes

Abbreviations

$[ATP]_i$	intracellular ATP concentration
DEND	neonatal diabetes with severe neurological complications
HetL164P	heterozygous L164P
HomL164P	homomeric L164P
K_{ATP}	ATP-sensitive K^+ channel

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NBD	nucleotide-binding domain
PNDM	permanent neonatal diabetes mellitus
P_o	channel open probability
SUR	sulfonylurea receptor

Introduction

ATP-sensitive potassium (K_{ATP}) channels link cellular metabolism to membrane electrical activity by regulating K^+ fluxes across the plasma membrane [1]. They are found in multiple tissues but are of particular importance in regulating insulin secretion from pancreatic beta cells [1, 2]. At substimulatory glucose concentrations, K_{ATP} channels are open so that the membrane potential is hyperpolarised and Ca^{2+} influx and insulin secretion are prevented [3]. Glucose metabolism enhances ATP production, resulting in closure of K_{ATP} channels, stimulation of electrical activity, opening of voltage-gated Ca^{2+} channels and exocytosis of insulin granules.

K_{ATP} channels are hetero-octamers of Kir6.x and sulfonylurea receptor (SUR) subunits [4–6]. Four inwardly rectifying subunits (Kir6.2 in pancreatic beta cells) form the pore of the channel and four auxiliary SUR subunits (SUR1 in pancreatic beta cells) associate with the tetrameric pore and regulate its gating [7–9]. Binding and/or hydrolysis of Mg-nucleotides by the intracellular nucleotide-binding domains (NBDs) of SUR produces channel opening [10–13]. It is believed that reciprocal changes in the intracellular concentrations of ATP and MgADP are involved in the metabolic regulation of K_{ATP} channels.

Over the last 4 years, many different missense mutations in the genes encoding Kir6.2 (*KCNJ11*) and SUR1 (*ABCC8*) have been shown to cause permanent neonatal diabetes mellitus (PNDM). This is a rare disorder characterised by high blood glucose levels that manifests within the first 6 months of life. A subgroup of mutations were associated with a more severe clinical profile characterised by Delayed development of motor, intellectual and social skills, muscle weakness, Epilepsy, facial dysmorphism and Neonatal Diabetes (DEND syndrome) [14–16]. Mutations in Kir6.2 have also been found to cause a remitting relapsing form of neonatal diabetes that resembles transient neonatal diabetes mellitus [17, 18].

All Kir6.2 mutations analysed to date were heterozygous and most were de novo mutations [14, 19–21]. In most cases, the diabetes they caused could be successfully treated with sulfonylureas [14, 20, 22], which directly close K_{ATP} channels by binding to the SUR1 subunit of the channel [23]. All PNDM mutations result in a reduced K_{ATP} channel sensitivity to inhibition by MgATP in vitro [15, 24]. This is expected to cause an increased K_{ATP} current amplitude and reduced insulin secretion. Studies to date suggest that the severity of the clinical phenotype reflects the extent of the reduction of the channel ATP sensitivity. Thus, mutations that produce a small increase in K_{ATP} current in the presence of physiological concentrations of MgATP (1–5 mmol/l) lead to PNDM, whereas mutations that cause a larger increase in K_{ATP} current give rise to DEND syndrome [15].

In this paper, we identify a *KCNJ11* mutation (L164P) that causes neonatal diabetes without obvious neurological complications. In functional studies, we show that the L164P mutation produces a large increase in the resting whole-cell current and a marked reduction in K_{ATP} channel sensitivity to inhibition by ATP. These effects are a secondary consequence of an increase in the channel open probability (P_o) produced by the mutation. Surprisingly, other mutations that produce a similar increase in P_o cause DEND syndrome. The L164P mutant channel was also far less blocked by the sulfonylurea tolbutamide, which explains why the patients were unable to transfer to glibenclamide therapy.

Methods

Participants Informed consent was obtained from all individuals investigated (or from their parents if they were children).

Molecular genetic analysis Genomic DNA was extracted from peripheral lymphocytes using standard procedures. The *KCNJ11* gene was amplified and sequenced as described [25]. Other family members were also tested for the novel mutation. Family relationships were confirmed using a combination of six microsatellites on chromosome 11: D11S902, D11S419, D11S1397, D11S1901, D11S921 and D11S1888.

Oocyte preparation Female *Xenopus laevis* were anaesthetised with ethyl 3-aminobenzoate methanesulfonate salt (MS222; 2 g/l added to the water). One ovary was removed via a mini-laparotomy, the incision sutured and the animal allowed to recover. Subsequently, animals were operated on for a second time, but under terminal anaesthesia. Immature stage V–VI oocytes were incubated for 60 min with 1 mg/ml collagenase (Type V; Sigma, Poole, UK) and manually defolliculated. All procedures were carried out in accordance with UK Home Office Legislations and the University of Oxford ethical guidelines. Oocytes were coinjected with ~0.8 ng wild-type or mutant Kir6.2 mRNA and ~4 ng mRNA encoding SUR. The final injection volume was 50 nl per oocyte. Isolated oocytes were maintained in Barth's solution and studied 1–4 days after injection.

Electrophysiology Wild-type or mutant Kir6.2 (GenBank D50581) were coexpressed with SUR1 in *Xenopus* oocytes (GenBank L40624) as described [26]. Because all the patients were heterozygous for the L164P mutation, their pancreatic beta cells will contain a mixture of wild-type and mutant Kir6.2 subunits. To simulate this heterozygosity, we coinjected *Xenopus* oocytes with a 1:1 mixture of mutant and wild-type Kir6.2 together with SUR1 mRNA. This is expected to give rise to a mixed population of channels composed of homomeric wild-type channels, homomeric mutant channels and heteromeric channels containing between one and three mutant subunits [21]. We refer to this global channel population as heterozygous channels.

Whole-cell currents were recorded using a two-electrode voltage clamp in response to voltage steps of ±20 mV from a holding potential of −10 mV, filtered at 1 kHz and digitised at 4 kHz. Oocytes were perfused with a solution containing (in mmol/l): 90 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$ and 5 HEPES (pH 7.4 with KOH). Metabolic inhibition was produced by 3 mmol/l sodium azide.

Macroscopic currents were recorded from giant inside-out patches using an EPC10 amplifier (List Medical Electronics, Darmstadt, Germany) controlled with Pulse v8.74 software (Heka Electronik, Lambrecht, Germany). Macroscopic currents were elicited by 3 s voltage ramps from −110 to +100 mV (holding potential 0 mV), or recorded at a constant potential of −60 mV. They were filtered at 0.5 kHz and digitised at 1 kHz. The pipette solution contained (mmol/l): 140 KCl, 1.2 $MgCl_2$, 2.6 $CaCl_2$, 10 HEPES (pH 7.4 with KOH). The internal (bath) solution contained (mmol/l): 107 KCl, 1 K_2SO_4, 2 $MgCl_2$, 10 EGTA, 10 HEPES (pH 7.2 with KOH) and Mg-nucleotides as indicated. Experiments were conducted at 20–22°C. Solutions were changed using a local perfusion system consisting of tubes of 200 μm diameter into which the tip of the patch pipette was inserted.

Nucleotide concentration–inhibition curves were fit with the Hill equation:

$$\frac{G}{G_c} = \frac{1}{1 + ([ATP]/IC_{50})^h}$$

where G is the K_{ATP} conductance in the presence of ATP, G_c is the K_{ATP} conductance in the absence of the nucleotide, [ATP] is the ATP concentration, IC_{50} is the nucleotide concentration at which inhibition is half maximal and h is the slope factor (Hill coefficient).

Single-channel currents were measured at −60 mV, filtered at 5 kHz and digitised at 20 kHz. Unitary amplitude and P_o were measured from the Gaussian fit to all-points amplitude histograms of tracts of current of 30–90 s duration.

Data were analysed with in-house routines developed in the IgorPro platform (Wavematrics, Portland, OR, USA). Data are given as means±SEM in the text and in the figures. Statistical significance was evaluated using a two-tailed Student t test and $p<0.05$ taken to indicate a significant difference.

Results

Patient characteristics and genetics Three unrelated probands with permanent neonatal diabetes were shown to be heterozygous for the *KCNJ11* gene mutation L164P (c.491T>C, p.Leu164Pro). All were female and had unaffected parents of different ethnic origin. Two patients have been reported previously [27, 28]. Mutation testing and microsatellite analysis of DNA from both parents and the child for two of the cases confirmed that the mutation had arisen de novo (the parents of the third case were not available for testing). None of the patients had any neurological complications or obvious developmental delay.

The first patient is currently 8 years old. She was born in Singapore from Sri Lankan parents, at 38 weeks of gestation with a birthweight of 2.6 kg. She developed diabetes at 30 weeks of age. When she came to Australia at the age of 6.5 years, her HbA_{1c} level was elevated (7.4%) and her diabetes required insulin treatment (0.45 U kg^{-1} day^{-1}). Glibenclamide treatment (1.0 mg kg^{-1} day^{-1}) was trialled for 2 months (at 7 years of age) but did not produce a decrease in her insulin requirement. She continues to require insulin (up to 0.7 U kg^{-1} day^{-1}) with an HbA_{1c} ranging from 7.2 to 8.2%. She has normal developmental milestones, her neurological examination is normal and she has no evidence of diabetic complications.

The second patient, born in Australia of Afghan origin, is currently 2.7 years old. She was born at term following an uneventful pregnancy, with a birthweight of 2.7 kg. She presented at 8 weeks in diabetic ketoacidosis and was subsequently treated (at 2.5 months for 4 weeks, then at 5 months for 3 months) with glibenclamide (up to 1.1 mg kg^{-1} day^{-1}, regimens of two times per day or three times per day), but had no reduction in insulin requirement (0.4 U kg^{-1} day^{-1}) [28]. Her mean HbA_{1c} since diagnosis has been 7.6% (range 7.2–8.7%). Assessment at 5 months by a neurologist was entirely normal, with a normal EEG. Subsequently, she has had normal developmental milestones and her neurological examinations have been normal at all follow up visits every 3 months. Diabetic complications have not been assessed due to the young age of the patient.

The third patient is from Slovakia [27]. She was born at term with a birthweight of 2.6 kg, and was diagnosed with diabetes at 5 weeks of age. She is currently 20 years old and is treated with insulin (1.24 U kg^{-1} day^{-1}). Glibenclamide transfer was not attempted because the patient also suffers from hepatitis C. She has poor glycaemic control, with an

HbA_{1c} level of 15.2%, and various diabetic complications including retinopathy and nephropathy. Psychomotoric development in childhood was normal. A neurological examination suggested the presence of diabetic motoric polyneuropathy in the lower extremities but there were no further neurological findings. Although her IQ was not tested, she successfully completed a specialised business school training suggesting normal mental development.

Functional analysis: effects on whole-cell K_{ATP} currents We examined the functional effects of the L164P mutation by heterologous expression in *Xenopus* oocytes. When wild-type Kir6.2/SUR1 channels are expressed in oocytes they are normally closed because they are inhibited by resting intracellular ATP concentrations ($[ATP]_i$). They can be opened, however, by azide (3 mmol/l; Fig. 1), a metabolic inhibitor that lowers $[ATP]_i$ [29]. Heterozygous L164P (hetL164P) currents were about 18-fold larger at rest than wild-type currents, but increased twofold on metabolic inhibition indicating that the channel is only partially closed at resting ATP levels (Fig. 1). In contrast, homomeric L164P (homL164P) channels displayed a much greater resting current and were little affected by metabolic inhibition (Fig. 1).

The sulfonylurea tolbutamide (0.5 mmol/l) blocked whole-cell K_{ATP} currents by 98% but had no effect on homL164P currents (4% block). HetL164P channels were blocked by only 54% (Fig. 1).

Functional analysis: effects on K_{ATP} channel ATP sensitivity The increase in resting whole-cell K_{ATP} currents suggests that the L164P mutation may reduce the channel ATP sensitivity, as found for other PNDM mutations [21].

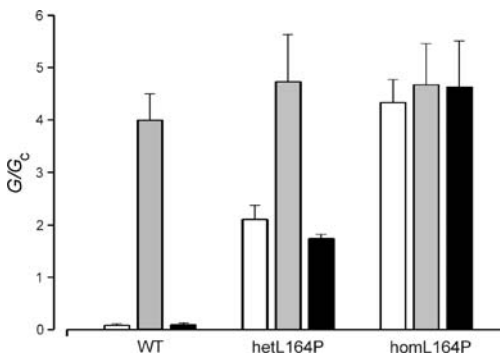

Fig. 1 Whole-cell K_{ATP} current. Mean steady state whole-cell currents evoked by voltage steps from −10 to −30 mV before (control, white bars) and after application of 3 mmol/l azide (grey bars) and in the presence of 3 mmol/l azide plus 0.5 mmol/l tolbutamide (black bars). The number of oocytes was five to seven in each case. *G*, K_{ATP} conductance; G_c, K_{ATP} conductance expressed relative to the conductance in the absence of the nucleotide. WT, wild-type; hetL164P and homL164P channels as indicated

We first measured the ATP sensitivity of wild-type and mutant K_{ATP} currents in the absence of Mg^{2+}, to isolate the effects of ATP on Kir6.2 (in the absence of Mg^{2+}, ATP does not interact with SUR1 [11]). Both homL164P and hetL164P channels had severely impaired ATP sensitivity. HomL164P channels were not blocked at all by ATP, even at concentrations as high as 10 mmol/l. The concentration–inhibition curve for hetL164P showed a striking shift to higher ATP concentrations, with an IC_{50} of about 100 μmol/l, and a marked pedestal of unblocked current at very high ATP concentrations. The data were best fitted by assuming that in the heterozygous state about 20% of channels are never closed by ATP (Fig. 2a,b and Table 1).

Molecular mechanism of the reduced ATP sensitivity Mutations that reduce the ATP sensitivity of the K_{ATP} channel can act in several ways. They may prevent ATP binding directly. They may impair the mechanism by which nucleotide binding is coupled to channel gating. They may also stabilise the intrinsic open state of the channel (i.e. that in the absence of ATP), which shifts the gating equilibrium in the presence of ATP towards channel opening and thus indirectly reduces the channel ATP sensitivity (e.g. [15, 30, 31]).

In a structural model of Kir6.2 [32], L164P lies within the channel pore, at a considerable distance from the ATP-binding site (Fig. 3). It is not predicted to interact directly with ATP. We therefore examined whether the L164P mutation alters intrinsic gating. Experiments were carried out in the absence of ATP, where intrinsic gating can be assessed. The L164P mutation had no effect on single-channel current amplitude (Table 2). However, the intrinsic P_o was markedly increased, being 0.86 (n=6) for homL164P compared with 0.4 (n=4) for wild-type channels (Fig. 4 and Table 2). This suggests that, at least in part, the L164P mutation alters channel ATP sensitivity indirectly, via an increase in P_o.

Effects on K_{ATP} channel ATP sensitivity in the presence of Mg^{2+} Previous studies have shown that Kir6.2 mutations associated with neonatal diabetes may not only decrease the sensitivity of Kir6.2 to ATP, but can also enhance channel activation by Mg-nucleotides [24]. We therefore explored the effect of the L164P mutation on the ATP sensitivity in the presence of 2 mmol/l Mg^{2+}.

Surprisingly, there was no difference in the effect of ATP in the presence and absence of Mg^{2+} on homL164P channels (Fig. 2c,d). This contrasts with what has been observed for mutations in the ATP-binding site that completely abolished ATP inhibition in the absence of Mg^{2+}, such as R50P and G334D: channels containing these mutations were activated by MgATP [33, 34]. The IC_{50} for ATP inhibition of hetL164P channels was also not significantly affected by Mg^{2+}, although the pedestal of unblocked current at high

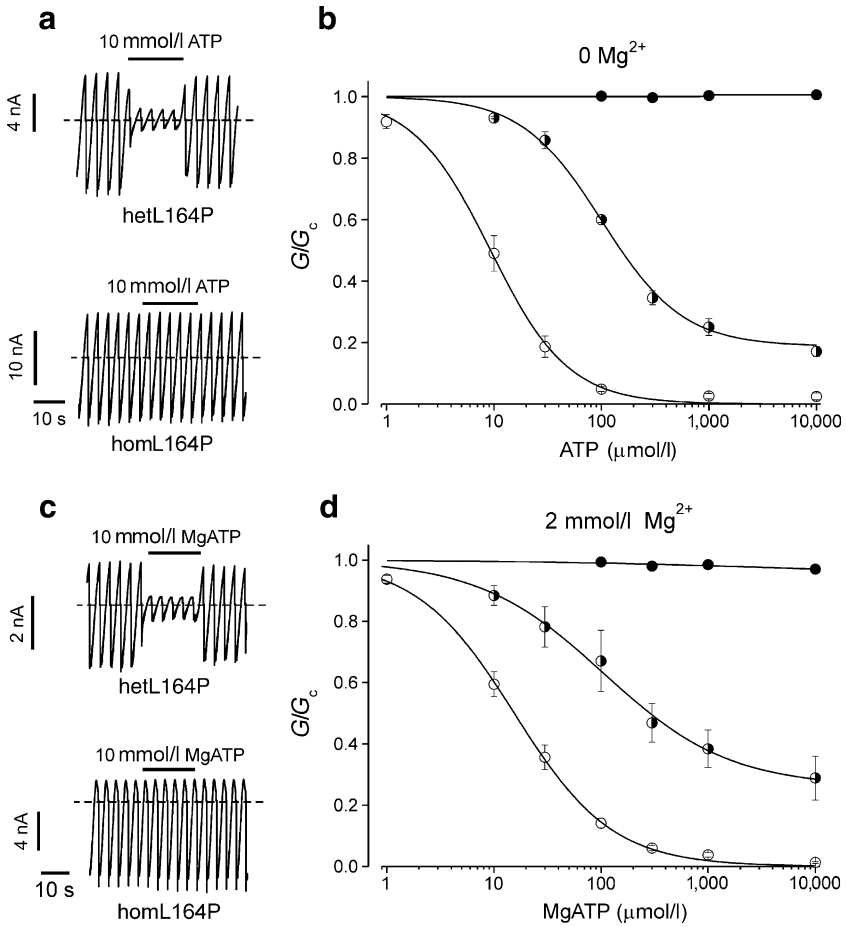

Fig. 2 ATP-inhibition of L164P channels is less that that of wild-type channels. **a**, **c** Currents recorded in inside-out patches excised from *Xenopus* oocytes expressing hetKir6.2-L164P/SUR1 (hetL164P) or homKir6.2-L164P/SUR1 (homL164P) channels, as indicated, in response to 3 s voltage ramps from −110 to +100 mV. ATP (10 mmol/l) was applied as indicated by the horizontal bars in the absence (**a**) or presence (**c**) of 2 mmol/l Mg^{2+}. **b**, **d**, mean relationship between [ATP] and K_{ATP} conductance (G), expressed relative to the conductance in the absence of the nucleotide (G_c), for wild-type (white circles, n=9), hetL164P (white/black circles, n=6) or homL164P (black circles, n=4) channels. Experiments were carried out in the absence (**b**) or presence (**d**) of 2 mmol/l Mg^{2+}. The continuous lines through the black circles were drawn by eye. The smooth curves are the best fit to the Hill equation with IC_{50} of 11 μmol/l (wild-type) and 100 μmol/l (hetL164P) (**b**, 0 mmol/l Mg^{2+}) or IC_{50} of 16 μmol/l (wild-type) and 122 μmol/l (hetL164P) (**d**, 2 mmol/l Mg^{2+})

ATP concentrations was increased by about 50% (Table 1). At 3 mmol/l MgATP, a concentration within the physiological range, the unblocked current was 34%, substantially greater than that found for wild-type channels (<1%; Table 1).

Functional analysis: effects on MgADP sensitivity The lack of MgATP activation of hetL164P channels could be due to reduced functional coupling between SUR1 and Kir6.2-L164P, or to reduced MgATP binding/hydrolysis at the

Table 1 ATP sensitivity of wild-type and mutant channels

	IC_{50} (Mg-free)	h (Mg-free)	IC_{50} (2 mmol/l Mg^{2+})	h (2 mmol/l Mg^{2+})	A (2 mmol/l Mg^{2+})	$\%I_{max}$ (3 mmol/l MgATP)
Wild-type	9.6±1.6	1.30±0.08	15.8±3.0	0.99±0.05	n.a.	0.01±0.01
hetL164P	100±8*	1.10±0.05	118±29*	1.16±0.34	0.29±0.07*	33.6±3.4*
homL164P	n.a.	n.a.	n.a.	n.a.	n.a.	98±1*

Values are means±SEM. The number of patches was four to nine in each case.
A The fraction of unblocked current used to fit the ATP concentration–inhibition relationship; *h* Hill coefficient; IC_{50}, ATP concentration (μmol/l) producing half-maximal inhibition; $\%I_{max}$ the per cent unblocked current in the presence of 3 mmol/l MgATP; *n.a.* not applicable (as no block).
*$p<0.05$ vs wild-type

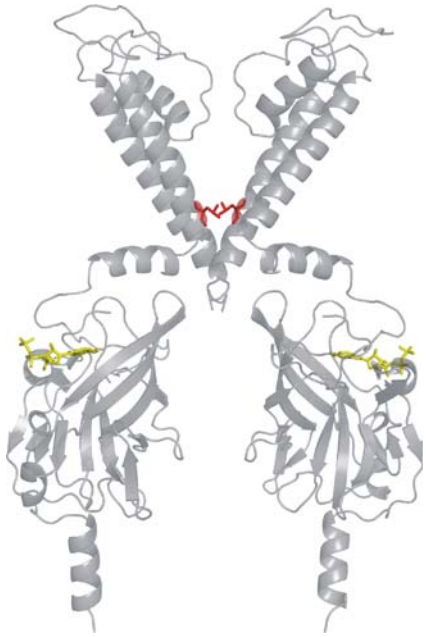

Fig. 3 Homology model of Kir6.2 [32]. For clarity, only two subunits are shown. ATP (yellow) is shown in its binding site. Residue L164 is shown in red

NBDs of SUR1. To explore the former possibility, we measured the ability of MgADP to activate hetL164P channels preblocked by 100 μmol/l MgATP. It was necessary to preblock the channels as the mutant channel has an open probability close to maximal and further activation is therefore not possible. Figure 5 shows that 30 μmol/l MgADP activates wild-type currents by 3.7±1.8 fold (n=3) and that 100 μmol/l MgADP did not produce any further increase (3.7±1.2-fold, n=3). In contrast, hetL164P channels were activated less: 1.4±0.1 fold (n=3) by 30 μmol/l MgADP and 1.5±0.1 fold (n=4) by 100 μmol/l MgADP.

Despite the fact that 100 μmol/l MgATP blocked mutant channels less, the lower extent of activation of hetL164P channels by 30 μmol/l MgADP is not due to the fact that channel activity is already maximal, as the amplitude is still significantly less than that in control solution (Fig. 5).

Table 2 Single-channel parameters for wild-type and homL164P channels

	P_o	i (pA)
Wild-type	0.39±0.05	4.0±0.1
homL164P	0.86±0.01*	4.1±0.1

Mean±SEM values of intrinsic open probability (P_o) and single-channel current (i) measured at −60 mV
The number of patches was five to six in each case
* p<0.05 vs wild-type

Fig. 4 The L164P mutation enhances single-channel activity. Representative single K_{ATP} channel currents recorded at −60 mV in inside-out patches from oocytes expressing wild-type or homL164P channels, as indicated. Currents were recorded in the absence of Mg^{2+} and nucleotides

Discussion

We describe the clinical and functional effects of a *KCNJ11* mutation, L164P, associated with permanent neonatal diabetes. This mutation causes a marked reduction in K_{ATP} channel inhibition by ATP primarily by stabilising the open state of the channel. This leads to an increase in the whole-cell K_{ATP} current, and in beta cells is expected to result in a reduction in insulin secretion. Surprisingly, unlike other mutations that reduced the channel ATP sensitivity by a similar amount, no motor or mental developmental delay was associated with the L164P mutation.

Structural considerations and molecular basis for reduced ATP sensitivity In a homology model of Kir6.2 [32], L164 lies partway along the permeation pathway, 35 Å away from the ATP-binding site (Fig. 3). It is therefore unlikely that it acts by reducing ATP binding directly. The side-chains of L164 point into the pore, forming a hydrophobic girdle that is narrow enough to prevent the passage of water and hydrated K^+ ions [35], which suggests that L164 may form a hydrophobic gate within the pore. Functional studies support this idea. Following mutation of L164 to cysteine, cadmium ions were able to block the K_{ATP} channel with high affinity [36, 37], suggesting that the four cysteines (one on each subunit) come together close enough to form a high-affinity binding site for Cd^{2+}. These data indicate that the side-chain of L164 must face into the pore, and that the pore is very narrow at this position. Substitution of a proline for L164 is expected to produce a kink in the α-helix [38] and disrupt the hydrophobic gate. It is therefore not surprising that the L164P mutation caused a dramatic effect on the P_o. Mutation of L164 to cysteine, alanine, valine, threonine or glycine also produces a very large increase in P_o [30, 36, 37, 39], consistent with the importance of this residue in channel gating.

The fact that the L164P mutation enhanced P_o can explain, in part, the reduced ATP sensitivity of the channel. It is also possible that the mutation may have effects additional to stabilisation of the channel open state.

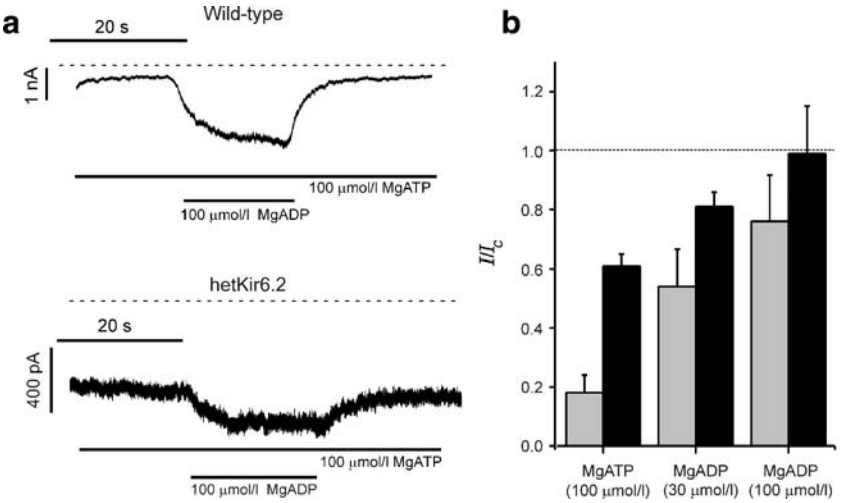

Fig. 5 Sensitivity to MgADP of wild-type and hetL164P channels. **a** Representative currents (*I*) recorded at −60 mV from inside-out excised membrane patches from *Xenopus* oocytes expressing wild-type or hetL164P channels, as indicated. Patches were exposed to 100 μmol/l ADP in the continuous presence of 100 μmol/l ATP: 2 mmol/l Mg^{2+} was present throughout. **b** Mean current in the presence of 100 μmol/l MgATP or 30 or 100 μmol/l MgADP plus 100 μmol/l MgATP, normalised to the current in the absence of nucleotides for wild-type (grey bars) and hetL164P (black bars) channels. Bars indicate means±SEM. The number of patches was three to four in each case

Effect of Mg^{2+} on Kir6.2-L164P/SUR1 channel ATP sensitivity In contrast to all PNDM mutations studied to date [24], MgATP was unable to enhance the activity of either homL164P or hetL164P channels. The lack of MgATP activation of homL164P channels may be due to the fact that P_o is already very high and thus there is no scope for further activation. However, little MgATP activation was also observed for hetL164P channels. This is in marked contrast to other mutations that caused enhanced P_o (I296L, [16], V59G [15]) where MgATP activated both homomeric and heterozygous channels. It is therefore possible that the L164P mutation reduces the efficacy of coupling between SUR1 and Kir6.2, and thereby decreases the ability of MgATP to stimulate channel activity. In support of this idea, MgADP activation of hetL164P channels was also reduced. This may explain the inability of Mg^{2+} to reduce ATP inhibition of hetL164P channels, since it is well established that MgATP must be hydrolysed to MgADP to stimulate channel activity.

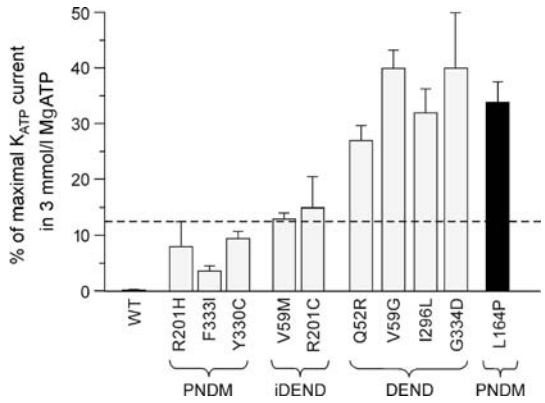

Fig. 6 Mean K_{ATP} current (expressed as a % of maximum) measured in the presence of 3 mmol/l MgATP from inside-out patches expressing heterozygous mutant channels as indicated. The dashed line indicates the maximal current amplitude normally associated with PNDM. iDEND, intermediate DEND syndrome (i.e. neonatal diabetes with developmental delay [21]). Data for wild-type (WT) and Kir6.2-R201H channels are from [14], for Y330C and F333I from [26], for Q52R, V59G and R201C from [15], for V59M from [24], for I296L from [16] and for G334D from [34]

The reason for the lack of Mg-nucleotide activation of hetL164P channels is unclear. Because L164 lies within the pore, far away from the NBDs of SUR1, the effect must be mediated allosterically. It is possible that this is mediated by an interaction between the backbone of L164 itself, or transmembrane domain 2 (within which L164 lies), and the transmembrane domains of SUR1. In the absence of an atomic resolution structure of the K_{ATP} channel complex, however, this cannot be definitely determined.

HomL164P channels were not blocked by tolbutamide, as expected because of their high P_o [40]. The lower efficacy of tolbutamide on hetL164P channels (about 50% block) may reflect the enhanced P_o of channels within the heterozygous population containing mutant subunits. It could also reflect impaired coupling between SUR1 and Kir6.2, such as that found for MgADP.

Clinical implications Previous studies of *KCNJ11* mutations have suggested that there is a good correlation between the percentage of current that remains unblocked in the presence of 3 mmol/l MgATP and the clinical phenotype. Namely, currents that are >30% of maximal are

associated with DEND syndrome, and those that lie between 5–10% of maximal with neonatal diabetes alone, compared with a current of <1% for wild-type channels (Fig. 6). The L164P mutation does not conform to this simple relationship between the functional effects of the mutation and the clinical phenotype. The magnitude of the hetL164P current in 3 mmol/l MgATP was 36%, yet none of the patients had extra-pancreatic symptoms.

The reason for this anomaly is not clear, particularly as the resting whole-cell current was also very large. Previous studies have also indicated that such large whole-cell currents are invariably associated with more severe clinical symptoms. The lack of responsiveness of the patient to sulfonylureas is consistent with the very large increase in P_o and the resting whole-cell current. It is therefore likely that hetL164P channels expressed in *Xenopus* oocytes are a reasonable model for the pancreatic beta cell K_{ATP} channels of the patients. We are therefore forced to conclude that compensatory mechanisms must ameliorate the extra-pancreatic effects of this mutation. The fact that the patients had very different ethnic origins suggests the compensatory mechanism is unlikely to reflect a shared genetic background: however, it does appear to be specific to the L164P mutation, which is unique (to date) in having a marked functional effect in vitro despite causing neonatal diabetes without neurological complications.

Importantly, a maximally effective concentration of tolbutamide only blocked hetL164P channels by 50%. All patients to date whose channels are blocked by <65% have not been able to transfer from insulin treatment to sulfonylurea therapy. This suggested that our patients would be unable to transfer to sulfonylurea therapy, as indeed was found to be the case. This is likely to be due to the fact that L164P destabilises the long closed state of the channel, to which sulfonylureas preferentially bind, and which is rarely entered in channels with enhanced P_o. Our results further suggest that not all patients with permanent neonatal diabetes will necessarily respond to sulfonylurea treatment, and indicates that knowledge of the functional effect of the mutation is helpful for predicting the drug response in patients.

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