Tropical Life Sciences Research, 20(1), 25–37, 2009

Mutational Analysis of The Er-Exit Code in The C-Terminal Domain of Kir6.2

[1]Vimala Devi Manickam, [1]Sugapriya Menaga Perumal, [2]Bharathiraja Chinnathambi and [2,3]Kannan Soundarapandian*

[1]Department of Biotechnology and Zoology, Ayya Nadar Janaki, Ammal College, Sivakasi 626124, Tamil Nadu, India
[2]Department of Ecobiotechnology, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India
[3]Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore 641046, Tamil Nadu, India

Abstrak: Mutasi pada gen yang mengekod subunit Kir6.2 pada saluran potassium, boleh mengakibatkan *congenital hyperinsulinism* (CHI). Mutasi pada CIH boleh memberi kesan yang teruk terhadap fungsi saluran dan proses keluar masuk dalam membran cel-β. Kajian ini melaporkan suatu mutasi baru E282K, dalam subunit Kir6.2 dalam seorang pesakit yang menghidapi CHI. Impak mutasi ini pada proses keluar masuk saluran dinilai melalui kajian immunositokimia dan fungsi saluran ditentukan oleh mutagenesis *site-directed*. Pengekspresan heterologous Kir6.2 dengan jelasnya menunjukkan bahawa mutasi di kod DXE telah mengakibatkan saluran kekal dalam ER. Kajian mutagenik yang selanjutnya telah menunjukkan bahawa E282K mungkin termasuk dalam kod exit DXE. Ini telah menyebabkan kemungkinan saluran K_{ATP} mungkin berkumpul bersama protin Sec 23/Sec 24, dan keluar dari ER dalam vesikel COPII, dalam satu proses yang memerlukan suatu bentuk Sar1-GTPase yang dominan negatif (iaitu tidak boleh menghidrolisasi GTP), dan menghalang saluran yang jenis liar keluar dari ER. Sebagai kesimpulan, saluran K_{ATP} menggunakan motif DXE untuk mengeksport protein-protein yang baru disintesis dari ER. Kepentingan fungsi kod ini didemonstrasikan oleh keterukan CHI, yang kelihatan seperti berpunca daripada mutasi gen di motif DXE. Maka, kajian ini telah menyediakan informasi baru iaitu Kir6.2 telah disintesis dalam ER, dan dieksport dengan bantuan motif DXE di dalam kawasan C-terminalnya.

Kata kunci: Diabetes mellitus, Saluran Potassium yang Sensitif ATP, Kir6.2, *Congenital Hyperinsulinism*

Abstract: Mutations in the gene encoding the potassium channel subunit Kir6.2 can cause congenital hyperinsulinism (CHI). CHI mutations severely affect channel function and trafficking in the β-cell membrane. The present study reports a novel mutation, E282K, in the Kir6.2 subunit in a patient diagnosed with CHI. The impact of this mutation on channel trafficking is assessed by immunocytochemical studies, and channel function is determined by site-directed mutagenesis. Heterologous expression of Kir6.2 clearly showed that mutations in the DXE code cause the channel to be retained in the ER. Further mutagenic studies revealed that E282K could be a part of the DXE exit code. This raised the possibility that K_{ATP} channels may assemble with Sec 23/Sec 24 proteins and exit the ER in COPII vesicles in a process that requires a dominant negative form of Sar1-GTPase (one that was unable to hydrolyse GTP) and prevented the ER exit of wild-type channels. This is due to the mutation in the C-terminal region which prevented the ER exit code. In conclusion, K_{ATP} channels use the DXE motif to export newly synthesized proteins from the ER. The functional importance of this code is demonstrated by the

* Corresponding author: skanmbt2008@yahoo.in

severity of CHI, which appears to be due to a genetic mutation in the DXE motif. Thus, the present study provides novel information that Kir6.2 is synthesized in the ER and exported with the aid of the DXE motif in its C-terminal region.

Keywords: Diabetes mellitus, ATP Sensitive Potassium Channel, Kir6.2, Congenital Hyperinsulinism.

INTRODUCTION

Diabetes is characterized by abnormally high sugar concentrations in the blood and urine that cause kidney and heart failure, blindness, and poor circulation. Diabetes is broadly classified into type 1 and type 2. Diabetes mellitus can be described as the result of an inadequate mass of functional pancreatic islet cells. The low insulin secretion seen in diabetes can be due to selective autoimmune destruction of β-cells as in type 1 diabetes, functional defects as in maturity onset diabetes of the young (MODY), or inadequate compensation for increased insulin demand due to insulin resistance as in type 2 diabetes. The present study focuses on type 2 diabetes, which is caused either by defective insulin secretion or impaired insulin action. Insulin is secreted by β-cells. These cells respond to rising levels of glucose in the blood by releasing insulin, which prevents hyperglycemia. Conversely when glucose levels fall below a particular threshold, β-cells stop secreting insulin, which prevents the equally dangerous hypoglycemia. Type 2 diabetes is a classic 'polygenic' disease caused by the interaction of multiple genes as well as the environment. However this is not always the case, as in 1%–2% of all cases a mutation in a single gene might be the potent factor for diabetes.

Ion channels are key molecules for signal transduction across biological membranes. Plasma membrane ion channels are easy to access and are often expressed at relatively low concentrations in specialized cells and tissues, like β-cells and Langerhans tissue (Niemeyer *et al.* 2001). K_{ATP} channels play a major role in regulating the membrane potential of pancreatic β-cells, and thereby induce the release of insulin (Reimann & Ashcroft 1999). In the absence of nutrients, K_{ATP} channels open and maintain the resting potential at around –70 mV. K_{ATP} channel closes in response to increased glucose metabolism, generating a membrane depolarization that activates voltage-gated Ca^{2+} channels. Thus Ca^{2+} influx and insulin release are quite possible. It is generally accepted that changes in ATP are the main physiological mechanism by which β-cell glucose metabolism regulates K_{ATP} channel activity (Ostenson *et al.* 2006).

K_{ATP} channels are involved in many physiological processes, including insulin secretion by pancreatic β-cells, regulation of vascular tone, and neuronal excitability. The Kir6.x subunits belong to the family of inwardly rectifying potassium channels. SUR is a member of the ATP-binding cassette (ABC) super family and contains 17 transmembrane helices, which are arranged in three clusters. SUR possesses two intracellular nucleotide-binding folds (NBFs), as well as binding sites for channel openers and sulfonylurea-type blockers (Aguilar-Bryan & Bryan 1999). The Kir and the SUR subunits of K_{ATP} channels are both

encoded by two genes, Kir6.1 and Kir6.2 and SUR1 and SUR2, respectively (Hambrock *et al.* 2002; Babenko & Bryan 2002). Kir6.2 and SUR1 are expressed in β-cells and islet α-cells, where they are involved in glucagon secretion (Donley *et al.* 2005; Hosy *et al.* 2007, Wang *et al.* 2007).

Mutations in the Kir6.2 gene affect insulin production by β-cells and reduce K_{ATP} channel activity, leading to increased insulin release and low blood sugar levels, a rare disorder called persistent hyperinsulinemic hypoglycemia of infancy (PHHI) (Hattersley & Ashcroft 2005). Loss-of-function mutations in both Kir6.2 and SUR1 cause congenital hyperinsulinism (De Vroede *et al*. 2004). Conversely, mutations in Kir6.2 that lead to reduced ATP sensitivity of the channel cause permanent neonatal diabetes, which in some cases is associated with muscle weakness, developmental delay, and epilepsy (Gloyn *et al*. 2004). In humans, activating mutations of KCNJ11 cause permanent neonatal diabetes.

Mutations in KCNJ11 cause CHI by either reducing or completely abolishing K_{ATP} channel activity at the surface membrane. Neonatal diabetes has the opposite phenotype of CHI (Porter & Barrett 2005). Inactivating mutations can give rise to over-secretion of insulin, and it has been hypothesized that activating mutations may result in the opposite phenotype to diabetes (Koster *et al.* 2005). In the KCNJ11 gene, a total of 32 mutations have been reported in families with HI, PNDM, DEND, and TNDM (Gloyn *et al.* 2004). CHI is the most important cause of persistent hypoglycaemia in the neonate and infant. It is a clinically and genetically heterogeneous entity. The clinical heterogeneity is manifested by severities ranging from an extremely severe life-threatening disease to very mild clinical symptoms that may even be difficult to identify. Two histopathological forms of CHI have been observed: a diffuse form and a focal form. Recent discoveries have begun to clarify the molecular etiology of the disease, and the mechanisms responsible for its clinical heterogeneity are becoming clear. The availability of cloned K_{ATP} channel genes allows for the characterization of this family of ion channels and identification of additional genetic defects (Lydia *et al.* 1998). Thus, the present study was designed to demonstrate that an ER-exit code is present in the C-terminal region of Kir6.2.

MATERIALS AND METHODS

Cell Lines and Plasmid Vectors

INS-1e (rat insulinoma) cells were a gift from Dr. G. Hardie (Dundee University, UK). TsA-201 cells, a modified form of HEK293 cells expressing a T-antigen, were a gift from Dr. A. Sivaprasatha Rao (University of Leeds, UK). DNA restriction and modification enzymes required for gene manipulation studies were purchased from Boehringer Mannheim and New England Biolabs (NEB). The mammalian expression vector pcDNA3.1 (+) and GFP were obtained from Invitrogen.

cDNA Clones

Mouse Kir6.2 (Genbank accession number D50581) was obtained from Dr. S. Seino (Osaka University, Japan) and was sub-cloned into pcDNA3.1. An HA epitope plus an 11 amino acid linker were inserted into the first extracellular loop to probe the surface-bound channels as described by Zerangue *et al*. (1999). E282K–EGFP was a kind gift from Dr. Sivaprasatha Rao (University of Leeds, UK). Sar1 constructs were a kind gift from Dr. W.E. Balch (Scripps Research Institute, USA). Myc-tagged GGA1 clones were obtained from Dr. David Beech (University of Leeds, UK). Tandem dimeric Kir6.2 constructs were produced by inserting an *EcoRI* site upstream of the stop codon of the Kir6.2 promoter 1 and upstream of the ATG start codon of the Kir6.2 promoter 2. Promoter 1 was then ligated into promoter 2 at the *EcoRI* site using T4 DNA ligase, thus generating the WT-WT tandem dimer. CHI mutants were introduced into promoter 2 to generate a tandem dimer of WT-mutant Kir6.2 subunits. Oligonucleotide primers designed for site-directed mutagenesis polymerase chain reaction (PCR) or DNA sequencing were purchased from Invitrogen Life Technologies. Primers were reconstituted in sterile deionised water to the concentration of 1nmol/l for long-term storage at –20ºC (Table 1).

Table 1: Oligonucleotide sequences used.

Mutant primer	Primer sequence (5'–3')
E282K FOR	ACCACCAGGACCTGAAGATCATTGTCATC
E282K REV	GATGACAATGATCTTCAGGTCCTGGTGGTG

Antibodies

Rat anti-HA antibodies (clone 3F10) were purchased from Roche Diagnostics. Mouse anti-Myc antibodies were purchased from Cell Signalling Technologies (UK). Alexafluor 593-conjugated antibodies and Alexa-488-transferrin were purchased from Molecular Probes Pvt. Ltd. Other FITC and Cy3-conjugated secondary antibodies were purchased from Jackson Immuno Research (USA). Mouse anti-CI-M6PR antibodies were purchased from Pharmacia Biotech India. Mouse anti-flag antibodies and anti-rat horseradish peroxidase (HRP) were purchased from Sigma.

QuikChange™ Mutagenesis

Site-directed mutagenesis was performed using the QuikChange™ method (Stratagene) and with specifically designed primers. Primers were 25–40 bases in length and had melting temperatures ~10°C above the standard extension temperature of 68°C. Melting temperatures were calculated using the formula:
$T_m = 81.5 + 0.41(\%GC) – 675/N – \%\ mismatch,$ where N is the primer length in base pairs, and %GC is the overall guanine and cytosine content. The desired mutation was placed in the centre of the primer with ~10–15 bases on each side. Each reaction mixture contained 1μl of DNA template (150 ng/μl), 1μl each of forward and reverse primers (10 mol/μl), 5 μl of 2 mM dNTP, 5μl of 10 X *Pfu* reaction buffer, 1μl of *Pfu* Turbo DNA polymerase (2.5 U/μl), and 36μl of Milli-Q.

Each reaction was overlaid with mineral oil (Hi-media), placed in a DNA thermal cycler (MJ Res. Inc. U.S.A), and subjected to the following parameters: 95°C for 30 seconds, followed by 55°C for 60 seconds, and 68°C. The duration of the elongation stage depended on the length of the template with each 1000 base pairs of template requiring 2 minutes of extension time. Once the 16 cycles were completed, the DNA was extended for 7 minutes at 72°C. Once the PCR was complete, 1μl of *DpnI* restriction endonuclease (20 U) was added, and the reaction was incubated for 1 hour at 37°C to digest the parental supercoiled DNA. The PCR products were then transformed into competent *E. coli* XL blue cells.

DNA Sequencing

All DNA sequencing was performed by Cambridge Gene Services (UK). The sequences of the primers used for DNA sequencing are shown in Table 2.

Table 2: Sequences of the primers used for DNA sequencing.

Primer name	Primer sequence (5'–3')
T7F	TAGTTATTGCTCAGCGGTGG
T7R	TAGTTATTGCTCAGCGGTGG
Kir6.2 C-terminus seq 1	GTGCAGAATATCGTCGGGCTG
Kir6.2 C-terminus seq 2	CGCTTTGTCCCCATTGTGGCC

Establishment and Maintenance of a HEK293 Cell Line Stably Expressing myc-Kir6.2

HEK293 cells stably expressing K_{ATP}-HA were cultured in Dulbecco's Modified Eagle's Medium (DMEM) medium supplemented with 10% foetal bovine serum (v/v), 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in a humidified atmosphere with 5% CO_2. HEK293 cells were transfected with pcDNA3.1-Kir6.2-HA+11aa-HMKFLAG using Fugene6 transfection reagent. After 48 hours, the culture media was supplemented with 800 μg/ml G418 and 10 μg/ml blasticidin to select for cells expressing both plasmids. After several days of continual treatment with G418 and blasticidin the remaining cells were re-plated at a low density and allowed to grow to form colonies. Individual colonies were cultured and assayed for expression of K_{ATP} channels. The clone displaying the optimal expression of K_{ATP} channels was then maintained in culture medium supplemented with 200 μg/ml G418 and 2 μg/ml blasticidin.

Immunocytochemical Methods

Staining of K_{ATP} channels at steady state

Cells were plated onto glass coverslips and transfected 36 hours before cell staining. Cells were fixed by addition of 3% paraformaldehyde (PFA) for 10 minutes, and when required the cell membranes subsequently permeabilised by addition of chilled 50% acetone:methanol for 10 minutes or 0.1% saponin in the

antibody-labelling media. For applications requiring selective surface labelling, i.e., when the cell membranes were left intact, the permeabilsation step was by-passed. Cells were then blocked with 5% goat serum in 1 X phosphate buffered saline (PBS) for 30 minutes, washed once with PBS, and incubated with the relevant primary antibodies in blocking medium for 2 hours at 25°C. Cells were then washed eight times with PBS and labelled with the appropriately fluorescent labelled antibody for 1 hour. The cells were then washed eight times in PBS, the coverslips were air dried on filter paper, and mounted onto microscope slides overlaid with VECTASHIELD mounting medium. The cells were then examined using a LSM confocal microscope. For co-labelling experiments, the cells were incubated separately with each primary antibody following by staining using the relevant secondary antibody. For relevant antibody concentrations see later sections.

Imaging fixed cells via confocal microscopy

Labelled cells were viewed using a Zeiss 510-META laser scanning confocal microscope (LSCM) under an oil-immersed 63× objective lens (NA = 1.40). FITC (494 nm excitation: 519 nm emission) was excited using an argon laser fitted with 488 nm filters, and Cy3 (550 nm excitation: 570 nm emission) was excited using a helium/neon laser fitted with 543 nm filters. The settings of each image were tailored so that the intensity of each pixel was within the range of sensitivity of the fluorescence detectors, i.e., no pixel showed saturation of fluorescence.

Recycling of K_{ATP} channels

HEK293 cells transfected with K_{ATP}-HA channels grown on a poly-lysine coated 24-well plate were incubated with 250 μl of 1:500 dilution of rat anti-HA antibody (0.2 μg/ml) for 2 hours at 37°C to label the endocytic pool. The cells were then washed with ice-cold DMEM and surface antibody was stripped *via* three 10 minute incubations in acid strip buffer containing 0.5M NaCl, 0.5% acetic acid (pH 3) at 4°C. The cells were then switched to pre-warmed blocking medium at 37°C for the indicated time periods to permit recycling of the labelled internalized channels. The cells were fixed with ice-cold 3% PFA, and after washing in PBS the cells were incubated with 250 μl of 1:500 dilution of goat anti-rat HRP for 1 hour at room temperature. After further washes the cells were dissolved in 400 μl of 2% sodium deoxycholate in PBS. Then chemiluminescence and the total protein content of the sample were evaluated. The initial reaction rates were normalized to the protein concentration for each time point and then normalized to the maximum signal, i.e., when recycling was at its peak. Recycling of mutant channels after 30 minutes was expressed relative to the wild-type recycling at the 30 minute time point. Experiments were done in duplicate.

RESULTS AND DISCUSSION

In the present study, we identified a new mutation, E282K, in the Kir6.2 subunit of a patient diagnosed with CHI. We examined the consequences of this mutation on channel trafficking and function using immunocytochemistry and Western blot

analysis. Kir6.2 was amplified with pcDNA3.1 (Fig 1). We next examined the mechanism of recycling with reference to the Kir6.2 protein (Fig 2). Kir6.2 protein was found to be synthesized in the endoplasmic reticulum and released into the cytosol after maturating in the trans Golgi network (TGN).

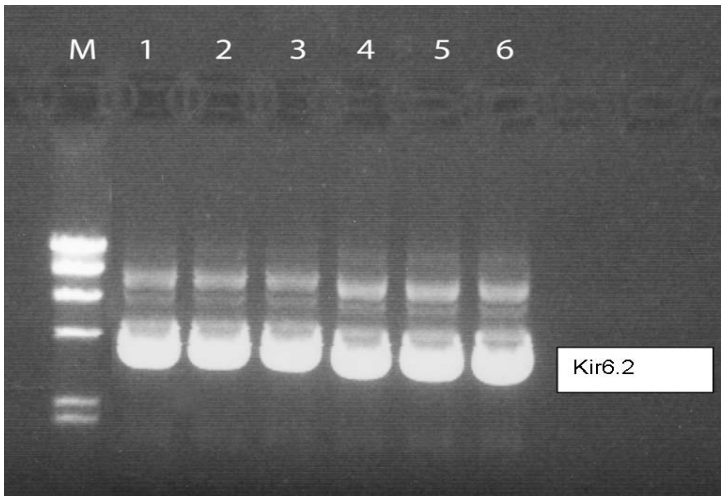
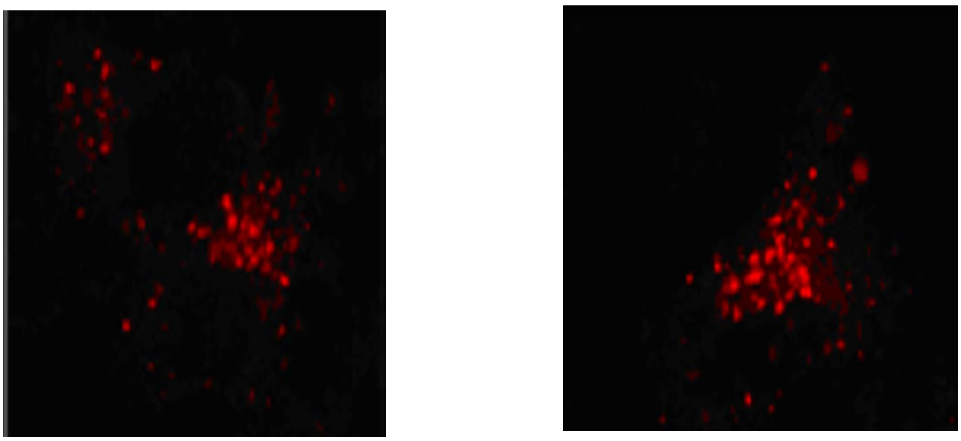


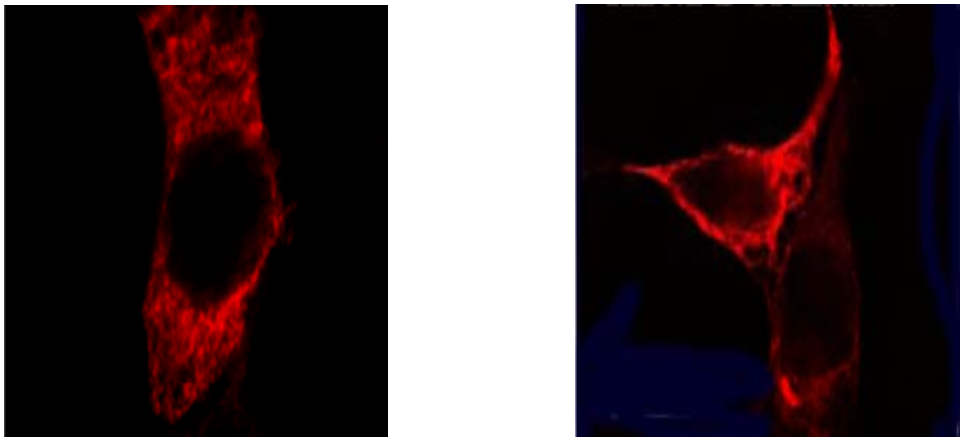
Figure 1: 0.9% agarose gel showing the PCR amplified product of Kir6.2 gene to be cloned into *E. coli* (XL-Blue) and the INS cell line.

Note: Lane 1, 2 and 3 shows the amplified Kir6.2 gene with plasmid DNA of clones of E coli (XL-Blue) strain
Lane 4, 5 and 6 shows the amplified Kir6.2 gene with plasmid DNA of GINS cells with clones of Kir6.2



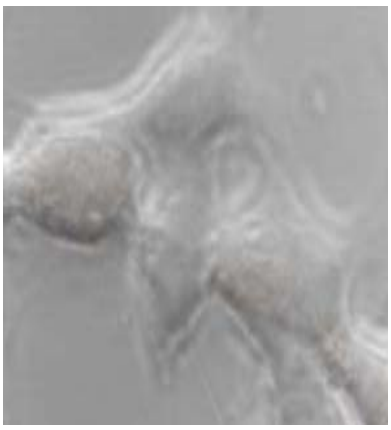
(a) (b)

Figure 2: (a) Confocal images illustrate the co-expression of GFP and HA-tagged Kir6.2. This membrane-bound receptor is visualized by treating the cells to goat anti-mouse HA (1:5000 dilutions); (b) the location of co-translocated Kir6.2 is examined using fluorescent microscopy, membrane-bound receptors are recycled back to the membrane.

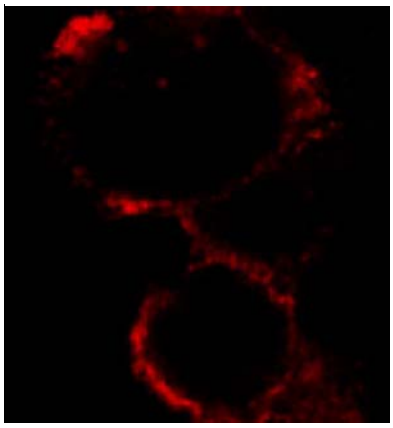


(a) (b)

Figure 3: (a) In control cells, Kir6.2 was expressed on the cell surface due to the wild-type DXE ER exit code; (b) in D-A mutants, the exit of Kir6.2 did not affect surface expression; (c) X-A mutants, surface expression was severely affected; (d) E-A mutants, surface expression was severely affected *(continued on next page).*



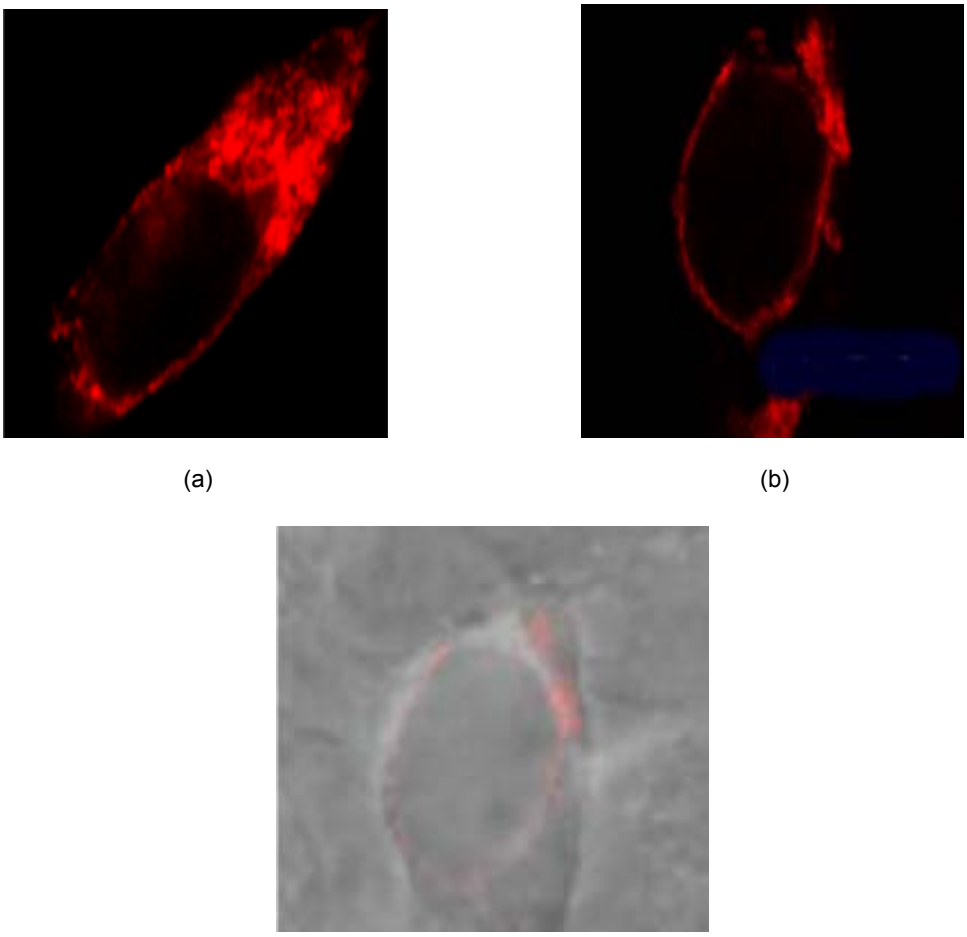
(c)



(d)

Figure 3: *(continued)*

The data presented in Figure 3 clearly showed that the site-directed mutation in the DXE motif severely affects the recycling of Kir6.2 with respect to the control. The effect of the triple mutant can be observed in Figure 4. Quantitatively, protein expression was found to be decreased in mutant cell lines when compared to control. Figure 5 shows the Western blot analysis that was carried out to study the co-expression of SUR and Kir6.2. The K_{ATP} channel protein was found to undergo maturation (glycosylation) in the TGN. Kir6.2 was impaired, which severely affects glycosylation. The DXE motif was identified as an ER exit motif. In this study, when the glutamic acid (E) was mutated to lysine (K), the expressed Kir6.2 protein did not mature, even in the presence of SUR1. By contrast, the presence of wild-type SUR1 and Kir6.2, along with mutated Sar1 (either H79G or T39N), did not allow for glycosylation to occur. Thus, the binding assay results clearly showed that Kir6.2 and SUR1, Kir6.2, SUR1 and Sar1 were able to effectively activate this post-translational modification. If the exit code of Kir6.2 or Sar1 was mutated, there was an inhibition of glycosylation. Thus, Kir6.2 E282K and Sar 1 H79G and T39N were identified as potential sites of ER exit motifs.



(a) (b)

(c)

Figure 4: (a) In Kir6.2-DXE control, confocal images show abundant surface expression of Kir6.2; (b) in Kir6.2-DXE mutant, surface expression of Kir6.2 was impaired in DXE mutants; (c) micrograph of a light image of the same DXE mutant.

Figure 5: Western blot analysis examining the maturation of SUR when genes involved in Kir6.2 maturation (i.e., glycosylation) were modified. Lane 1, Sur1 was not able to mature due to the absence of Kir6.2. Lane 2, the presence of both Sur1 and Kir6.2 allow SUR to mature. Lane 3, the presence of wild-type Sar1 further enhanced protein maturation. Lane 4, even in the presence of SUR, Kir6.2 protein did not mature due to the Kir6.2 E282K point mutation and the absence of wild-type Sar1. Lane 5, wild-type SUR1, wild-type Kir6.2, and Sar1 H79G did not allow for protein maturation. Lane 6, mutant Sar1 (T39N) also effectively inhibited the protein maturation process in the presence of wild-type Kir6.2 and SUR. Protein bands located on the bottom row are immature proteins (non-glycosylated) whereas on top row are fully matured proteins (glycosylated).

In the present study, immunocytochemistry and electrophysiology were used to examine the effects of the mutation on ion channel trafficking and function, respectively. Gloyn *et al.* (2004) found that arginine (Arg)-based ER localization signals are involved in the control of different heteromultimeric membrane protein complexes. Heteromultimeric complex assembly is controlled by a checkpoint mechanism based on ER localization motifs present in both Kir6.2 and SUR subunits (Zerangue *et al.* 1999). This Arg-based peptide retention signal prevents unassembled subunits or partially assembled complexes from reaching the cell surface. By employing an affinity purification approach, they showed that the Arg-based ER localization signal (RKR) present in the cytoplasmic C-terminus of Kir6.2 is recognized by the coat protein I (COPI) vesicle coat as well as 14-3-3 proteins. COPI binding can explain the ER localization activity of the RKR motif. In this study, western blot analysis of the co-expression of SUR and Kir6.2 revealed that the K_{ATP} channel protein was found to be undergoing maturation (glycosylation) in the TGN. If any one of the two genes were impaired the glycosylation process was seriously affected.

SUR1, when expressed alone has a long life, whereas when Kir6.2 and SUR1 are co-expressed, they associate rapidly and the fast degradation of Kir6.2 is eliminated. Based on the glycosylation state of SUR1, the half-life for the maturation of K_{ATP} channel including completion of assembly, transit to the Golgi, and glycosylation is ~2.2 hours. Zerangue *et al.* (1999) observed that when expressed alone, Kir6.2 and SUR1 are retained within the ER due to their Arg-based signals. In the absence of SUR1, Kir6.2 can still form tetramers but these are retained inside the cell, indicating that co-assembly with other Kir6.2 subunits

cannot mask the Arg-based signals. Only when SUR1 is present is Kir6.2 able to reach the membrane, which implies that inactivation of the Arg-based signal is a consequence of co-assembly with SUR1. The simplest explanation is that the RKR motif of Kir6.2 is sterically masked in the presence of SUR, thereby preventing COPI binding. The DXE motif was identified as an ER exit code. In this study, when glutamic acid was mutated to lysine, the protein did not mature, even in the presence of GTP and GDPase activity. H79G prevents GTPase activity whereas T39N mutation blocks the GDP activity.

Thus, the present study explains the physiological and biochemical importance of the DXE motif. We demonstrated that the DXE motif is one of the most significant sites for determining the transport of mature Kir6.2 from the ER to the Golgi complex. We observed that mutation at DXE led to impairment of the ER-exit code, and the protein was not able to be recycled back to the membrane. This molecular defect leads to several diseases, such as transient neonatal diabetes mellitus (TNDM), permanent neonatal diabetes mellitus (PNDM), congenital hyperinsulinism (CHI), severe developmental delay epilepsy, permanent neonatal diabetes (DEND) syndrome, and persistent hyperinsulinemia and hypoglycemia of infancy (PHHI) (Gloyn *et al.* 2004). Thus, this preliminary attempt helps to elucidate the molecular mechanism of these genetic diseases and could pave the way to find suitable remedies for such genetic disorders.

ACKNOWLEDGEMENT

The authors are very thankful to Ayya Nadar Janaki Ammal College, Sivakasi for providing an excellent laboratory facility. The corresponding author would like to thank the Department of Biotechnology (DBT), Government of India for providing the opportunity to undergo research training in this field in the United Kingdom.

REFERENCES

Aguilar-Bryan L and Bryan J. (1999). Molecular biology of adenosine triphosphate-sensitive potassium channels. *Endocrine Reviews* 20: 101–135.

Aguilar-Bryan L, Clement IV J P, Gonzales G, Kunjilwar K, Babenko A and Bryan J. (1998). Toward understanding the assembly and structure of K_{ATP} channels. *Physiological Reviews 78*(1): 227–245.

Babenko A P and Bryan J. (2002). Sur-dependent modulation of K_{ATP} channels by an N-terminal Kir6.2 peptide: Defining intersubunit gating interactions. *Journal of Biological Chemistry* 277(46): 43997–44004.

De Vroede M, Bax N M A, Brusgaard K, Dunne M J and Groenendaal F. (2004). Laparoscopic diagnosis and cure of hyperinsulinism in two cases of focal adenomatous hyperplasia in infancy. *Pediatrics* 114: 520–522.

Donley V R, Erin K H A, Kidder A C and Schermerhorn T (2005). ATP-sensitive potassium channel (K_{ATP} channel) expression in the normal canine pancreas and in canine insulinomas. *BMC Veterinary Research* 1.

Gloyn A L, Pearson E R, Antcliff J F, Proks P, Bruining G J, Slingerland A S, Howard N *et al.* (2004). Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. *New England Journal of Medicine* 350(18):1838–1849.

Hambrock A, Preisig-Müller R, Russ U, Piehl A, Hanley P J, Ray J, Daut J, Quast U and Derst C. (2002). Four novel splice variants of sulfonylurea receptor 1. *American Journal of Physiology: Cell Physiology* 283: C587–C598.

Hattersley A T and Ashcroft F M. (2005). Activating mutations in Kir6.2 and neonatal diabetes: New clinical syndromes, new scientific insights and new therapy. *Diabetes* 54(9): 2503–2513.

Hosy E, Derand R, Revilloud J and Vivaudou M. (2007). Remodelling of the SUR-Kir6.2 interface of the K_{ATP} channel upon ATP binding revealed by the conformational blocker rhodamine 123. *Journal of Physiology* 582(1): 27–39.

Koster J C, Alan Permutt M and Colin G. (2005). Diabetes and insulin secretion: The ATP-sensitive K^+ channel (K_{ATP}) connection. *Diabetes* 54: 3065–3072.

Lydia A, John P, Clement J, Gonzalez G, Kunjilwar K, Babenko A and Bryan J. (1998). Toward understanding the assembly and structure of K_{ATP} channels. *Physiological Reviews* 78(1): 227–245.

Niemeyer B A, Mery L, Zawar C, Suckow A, Monje F, Pardo L A, Stühmer W, Flockerzi V and Hoth M. (2001). Ion channels in health and disease. 83rd Boehringer Ingelheim Fonds International Titisee Conference. *EMBO Reports* 2(7): 568–573.

Ostenson C-G, Gaisano H, Shew L, Tibell A and Bartfai T. (2006). Impaired gene and protein expression of exocytotic soluble N-ethylmaleimide attachment protein receptor complex proteins in pancreatic islets of type 2 diabetic patients. *Diabetes* 55: 435–440.

Porter J R and Barrett T G. (2005). Monogenic syndromes of abnormal glucose homeostasis: Clinical review and relevance to the understanding of the pathology of insulin resistance and beta cell failure. *Journal of Medical Genetics* 42(12): 893–902.

Reimann F and Ashcroft F M. (1999). Inwardly rectifying potassium channels. *Current Opinion in Cell Biology* 11: 503–508.

Wang R, Su J, Zhang X, Shi Y, Cui N, Onyebuchi V A and Jiang C. (2007). Kir6.2 channel gating by intracellular protons: Subunit stoichiometry for ligand binding and channel gating. *Journal of Membrane Biology* 213(3): 155–164.

Zerangue N, Schwappach B, Jan Y N and Jan L Y. (1999). A new ER trafficking signal regulates the subunit stoichiometry of plasma membrane K (ATP) channels. *Neuron* 22:537–548.