The beta-cell Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II) β3 isoform containing a proline-rich tandem repeat in the association domain can be found in the human genome

To the Editor: Since the multifunctional Ca^{2+} calmodulin-dependent protein kinase II (CaM kinase II) is expressed in the pancreatic beta cell and is activated by glucose and other secretagogues in a manner correlating with insulin secretion, it has been proposed that CaM kinase II mediates some of the actions of Ca^{2+} on the exocytosis of insulin [1]. There are four distinct genes, *CAMK2A, CAMK2B, CAMK2G* and *CAMK2D,* which code for four CaM kinase II isoforms α,β,γ and δ respectively. Alternatively spliced variants of each gene, which differ in insertions or deletions between the calmodulin binding site in the regulatory domain and the association domain, are expressed in different tissues [2].

A splice variant of the *CAMK2B* gene denoted CaM kinase Il β3 was identified as a novel pancreatic beta-cell isoform by cloning from a neonatal rat islet cDNA library [3]. The authors subsequently confirmed this finding in beta-cell lines and testis by DNA amplification of the sequence encoding the inserted domain by reverse transcriptase-polymerase chain reaction (RT-PCR] followed by Southern analysis. The β3 isoform is predicted to be a protein of 589 amino acids containing a unique 86 amino acid insertion in the association domain (corresponding to nucleotides 1155–1412). This insertion comprises two repeated motifs of 43 amino acids each, in a tandem arrangement, which are 84 % identical. These repeats are proline rich (27 %) and hydrophobic (84 %). However, in a recent study on human tissues, cloning and quantitative measurement of CaM kinase II isoforms indicated that three different splice variants of the *CAMK2B* gene, termed βl, β2 and β4, were expressed in beta cells and other endocrine tissue such as adrenals and pituitary, but the authors were not able to identify the CaM kinase II β3 isoform in any of the primary tissues (endocrine tissues, liver, adipose tissue and lymphocytes) investigated [4]. Moreover investigators were unable to detect a human CaM kinase II β3 isoform by PCR amplification of the variable region [5]. These findings cast doubt on the existence of the CaM kinase II β3 variant in humans. Near completion of sequencing of the human genome has allowed us to resolve this issue.

We searched the NCBI human genome database for the genomic sequence of the *CAMK2B* gene and to determine the genomic structure. Using the published cDNA sequence for the rat CaM kinase II β3 isoform (GenBank accession number X83 375) we carried out BLAST searches of the database. Homology searches carried out using the published cDNA sequences of various β isoforms confirmed that *CAMK2B* is located on chromosome 7. A 153 kilobase contig on chromosome 7 (Accession Number NT_002 349.1) was found to contain 15 exons which occur in the β3 isoform and to account for all except the first 382 nucleotides at the 5'-end of the cDNA sequence. There was a high degree of homology between the coding sequence of the rat and human genes which we have also observed with other CaM kinase II genes [6]. The two parts of the proline rich insertion domain are clearly in two separate exons over 2000 bp apart. lnterestingly a third exon coding for a homologous proline-rich domain is also present in the human *CAMK2B* gene.

The proline rich domain of the CaM kinase II β3 isoform conforms to an SH3-binding sequence and therefore could be involved in targeting CaM kinase II directly to subcellular sites and/or substrates. Another possibility is that the domain could influence the oligomeric nature of the enzyme which has been shown to exist as a heteromultimer or homomultimer of between 8 to 12 subunits [2].

We have previously provided evidence for expression of the β3 splice variant in human islets of Langerhans. Using RT-PCR of human islet poly A^+ mRNA with primers based on the sequence of the unique proline-rich domain we amplified a 303 bp product identical in sequence to that reported for rat islet β3 isoform of CaM kinase II [7]. We subsequently obtained evidence by western blotting for expression of CaM kinase II β3 protein in human islets of Langerhans [8]. Our observations provide the genetic basis for these studies by showing that the CaM kinase II β3 isoform is indeed encoded by the human *CAMK2B* gene on chromosome 7 with the proline rich domain comprising two widely separated exons. The expression of an isoform with a putative domain involved in targeting specific subcellular organelles is of considerable interest given the strong evidence for involvement of CaM kinase II in the control of insulin secretion [1].

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References

1. Easom RA (1999) CaM kinase Il: a protein kinase with extraordinary talents germane to insulin exocytosis. Diabetes 48: 675–841
2. Braun AP, Schulman H (1995) The multifunctional calcium/calmodulin-dependent protein kinase: From form to function. Annu Rev Physiol 57: 417–445
3. Urquidi V, Ashcroft SJH (1995) A novel pancreatic beta-cell isoform of calcium/calmodulin-dependent protein kinase 11 (beta3 isoform) contains a proline-rich tandem repeat in the association domain. FEBS Lett 358: 23–26
4. Rochlitz H, Voigt A, Lankat-Buttgereit B et al. (2000) Cloning and quantitative determination of the human Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II) isoforms in human beta cells. Diabetologia 43: 465–473
5. Tombes RM, Krystal GW (1997) Identification of novel human tumour cell-speciftc CaMK-II variants. Biochim Biophys Acta 1355: 281–292
6. Gloyn A, Hashim Y, Ashcroft SJH. (2000) Determination of the genomic structure of the calcium/calmodulin protein kinase Il gamma gene, a candidate gene for Type II diabetes. Diabetologia 43 [Suppl 1]: A82 327
7. Breen MA, Ashcroft SJH (1997) Human islets of Langerhans express multiple isoforms of calcium/calmodulindependent protein kinase II. Biochem Biophys Res Commun 236: 473–478
8. Breen MA, Ashcroft SJH (1997) A truncated isoform of Ca^{2+} /calmodulin-dependent protein kinase expressed in human islets of Langerhans may result from trans-splicing. FEBS Lett 409: 375–379

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