

## Calcium/calmodulin dependent Protein Kinase II genes: genomic structure and screening for variants in subjects with type 2 diabetes

A.L. Gloyn, M. Desai, A. Clark, R.R. Holman, T.M. Frayling, A.T. Hattersley, S.J.H. Ashcroft

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**Background and Aims:** CaM Kinase II, a multifunctional Ca<sup>2+</sup>/calmodulin-dependent protein kinase, 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 the activation of CaM Kinase II mediates some of the actions of Ca<sup>2+</sup> on the exocytosis of insulin. Thus, the genes encoding for members of this multigene family are important candidate gene for beta-cell dysfunction in Type 2 diabetes (T2DM). We aimed to determine the genomic structure of both the gamma and delta isoforms (*CAMK2G* and *CAMK2D*) and screen the exons and exon-intron boundaries for variants in subjects with T2DM.

**Materials and Methods:** Genomic structure of the *CAMK2G* gene was determined by identifying 6 contiguous clones from a human P1 artificial chromosome (HPAC) library using a CaM Kinase II  $\gamma$  cDNA probe. Positive clones were confirmed as  $\gamma$  by PCR amplification of the  $\gamma$  specific variable domain VIII. Fluorescence *in situ* hybridisation (FISH) localised these clones to chromosome 10q22. The published genomic structures of the rat and mouse CaM Kinase II genes allowed the putative exon-intron boundaries of human *CAMK2G* to be identified. These were confirmed by vectorette PCR amplification and resequenced from genomic DNA. *CAMK2D* genomic structure was determined by sequence homology to genomic contigs in the NCBI database. DNA from 76 randomly selected subjects with T2DM was screened by SSCP analysis for variants in both genes.

**Results:** *CAMK2G* was composed of 23 exons (43-123 bp) whilst, *CAMK2D* consisted of 18 exons (42-122 bp). Screening of both genes has identified variants; in *CAMK2G* a silent variant ( $K^{49}K$ ) in exon 2 (AAA→AAG) which encodes for part of the catalytic domain and an intronic variant in exon 10 (+58c→t). In *CAMK2D* an intronic variant was detected in exon 14 (-45 g→a).

**Conclusions:** We have determined the genomic structures of *CAMK2G* and *CAMK2D*, localised the *CAMK2G* gene to chromosome 10q22 and identified variants which can be used to determine the role of these genes in susceptibility to T2DM.