

Isolation of genes regulating insulin secretion in the *Niddm1i4*-locus in the GK-rat by functional genomic- and physiological-analysis

Aims

1. To investigate the possible roles of candidate genes in the *Niddm1i4* locus in the GK rat that encodes hyperglycemia, hypoinsulinemia, and defective insulin exocytosis.
2. To identify the molecular mechanisms behind the *Niddm1i4* locus' impact on glucose homeostasis.

Introduction

Type 2 diabetes is defined by chronic elevation of plasma glucose, but the underlying pathophysiology is complex and profoundly influenced by both environmental factors and polygenic background. In a series of experiments a major genetic locus (*Niddm1*) on the telomeric end of rat chromosome 1 was discovered. This locus explains 30% of the genetic glucose variance in F2-intercrosses between GK and normoglycemic F344 rats. Studies of the congenic strain NIDDM1I demonstrated that *Niddm1i*^{GK} encoded hyperglycemia, and insulin secretion defects in isolated pancreatic islets. *Niddm1i* encodes an insulin deficient type of hyperglycemia. To finemap *Niddm1i*, high resolution linkage analysis and analysis of congenic strains was combined. This approach identified four subloci mapped to intervals less than 1 million base pairs in the *Niddm1i* region. For two of these subloci, the genes behind the diabetes phenotype are identified, *Sorcs1* and *Sorcs3*. The cellular phenotype of the other two statistically proven loci was also characterized. One, *Niddm1i1* encodes an energy deficiency phenotype (low ATP/ADP in islets); the other, *Niddm1i4*, encodes an isolated insulin secretion defect.

Fine-mapping located one hyperglycemia locus, *Niddm1i4* ($P=5 \times 10^{-6}$), which covers a 700 kb genome interval with seven identified genes, including the gene for programmed cell death 4 (*Pdcd4*), three genes with unknown functions, one microRNA gene, the leucine-rich repeat protein SHOC2 and the α -2-adrenergic receptor. *Niddm1i* is homologous to both human and mouse regions linked with type 2 diabetes-susceptibility, therefore studies in animal models can reveal information valuable for understanding human diabetes.

Preliminary data demonstrate that the *Niddm1i4* locus is even more complex and code for several diabetes associated phenotypes (insulin and glucose concentration as well as insulin and exocytosis in isolated islet cells). Therefore, this work will seek to identify additional genes responsible for the complex diabetes phenotype.

Research plan

With this work aim to identify functions of *Niddm1i4* at the genetic, molecular, cellular, and *in vivo* levels. We are going to use experimental animals both diabetic GK/Swe, normoglycemic F344 /DuCr12Swe, and congenic strains derived from them. Three congenics (N1I5, 7, and 11 cover the *Ni4* and display different phenotypes, strongly indicating at least two subloci within the locus.

To avoid effects of the estrus cycle and other minor gender specific influences, only males will be included in this study. Weight measurements and an intraperitoneal glucose tolerance test with 2.0 g glucose/ kg body weight will be performed after 6 h fasting at 95 days of age. Insulin secretion and exocytosis from isolated islets from new subcongenic strains will be characterized. All genes in the QTL will be sequenced and their RNA expression will be determined in pancreas and other relevant tissues. For those with aberrant expression and/or major DNA sequence

variation between GK and F344, antibodies will be raised against synthetic peptides. Extensive analysis of the transcriptomes (Affymetrix) of different congenics will allow identification of the different pathways involved and establish correlations to functional and morphological phenotypes.

Methods and techniques:

1. An intercross between the two most extreme congenics covering the *Nli4* locus will be established to generate new subcongenic lines in order to further refine the genetic map of functional defects at the whole-body and cellular levels encoded by the locus. The locus covers approximately one cM, so several new recombinants will be feasible to obtain from 3-500 F2 offspring.
2. Phenotypic and genotypic evaluation and characterization of new recombinants. Several markers (microsatellites and SNPs) are already available within the locus and DNA sequencing will identify additional markers.
3. Islet isolation from subcongenic strains will be used to determine insulin secretion and exocytosis phenotypes as well as the ultrastructural phenotype (electron microscopy).
4. All genes in the defined QTL will be sequenced and their RNA expression patterns as well as complete transcriptomes (Affymetrix micro arrays) will be determined in pancreas and additional relevant tissues in collaboration with other nodes in the Nordic network.
5. Real-time PCR for quantification of transcripts within the QTL.