Polymorphisms at the GLUT1 (HepG2) and GLUT4 (muscle/adipocyte) glucose transporter genes and non-insulin-dependent diabetes mellitus (NIDDM)

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Summary. In order to determine the possible contribution of the GLUT1 (HepG2) glucose transporter gene to the inheritance of non-insulin-dependent diabetes mellitus (NIDDM), two restriction fragment length polymorphisms (RFLPs) and the related haplotypes at this locus were studied in 48 Italian diabetic patients and 58 normal subjects. Genotype frequencies for the XbaI polymorphism were significantly different between patients and controls ($X^2 = 9.80$, $df = 2$, $P < 0.0079$). A significant difference was also found in the allele frequencies between NIDDM patients and controls ($X^2 = 9.39$, $df = 1$, $P < 0.0022$), whereas no differences were found for the StuI RFLP. No linkage disequilibrium was detected between the XbaI and StuI RFLPs in this sample. The analysis of the four haplotype frequencies ($XIS1$, $X1S2$, $X2S1$, $X2S2$) revealed a significant difference between diabetic patients and controls ($X^2 = 14.26$, $df = 3$, $P < 0.002$). By comparing single haplotype frequencies, a significant difference between the two groups was found for the $X1S1$ and $X2S2$ haplotypes. A two-allele RFLP at the GLUT4 (muscle/adipocyte) glucose transporter gene, detected with the restriction enzyme KpnI, was also examined; no differences were found between patients and controls for this RFLP. The finding of an association between polymorphic markers at the GLUT1 transporter and NIDDM suggests that this locus may contribute to the inherited susceptibility to the disease in this Italian population.

Introduction

Genetic factors contribute a major part to the aetiology of non-insulin-dependent diabetes mellitus (NIDDM). The greater than 90% concordance rate for the disease among monozygotic twins (Barnett et al. 1981) provides strong evidence for a major genetic component, but the gene (or genes) involved are still unknown. Many metabolic defects have been demonstrated in NIDDM, including defects in the binding of insulin to its receptor (Olefsky and Reaven 1974), reduced tyrosine-kinase activity of the insulin receptor β-subunit (Comi et al. 1987; Freidenberg et al. 1987), and impaired glucose transport activity (Kashiwagi 1983; Ciaraldi et al. 1982).

The distinction between inherited defects and defects secondary to metabolic abnormalities can be difficult to elucidate. One method for identifying genetic factors in polygenic disorders such as NIDDM is to use restriction fragment length polymorphisms (RFLPs) as markers for candidate genes and to examine the differences in genotype and allele frequencies between diabetic patients and matched control subjects. Such differences may be demonstrated because of linkage disequilibrium between the alleles at the RFLP site and the alleles involved in the susceptibility to the disease. Candidate genes that have been considered to contribute to the aetiology of NIDDM include the insulin gene, the insulin receptor gene and the glucose transporter genes. Evidence from pathophysiological data suggests the presence of inherited defects of glucose transport, in cases where a reduction in both glucose transporter numbers and activity in adipocytes is observed and when a reduced sensitivity to the action of insulin that is not reversible after insulin treatment is found (Kashiwagi 1987; Ciaraldi et al. 1982; Garvey et al. 1988). Furthermore, a decrease in glucose oxidation in NIDDM patients has been reported (Golay et al. 1988); it has been suggested that a defect in glucose transport may account for this (Butler et al. 1990).

Four different glucose transporter proteins have been recently identified, each of them expressed by a particular gene and considered to have a specific tissue distribution (Mueckler 1990). The best characterized of the glucose transporter genes is the GLUT1 or HepG2 glucose transporter gene (Mueckler et al. 1985); this gene is present on chromosome 1p31.3–p35 (Show et al. 1987), is ubiquitously expressed, and is the major transporter in brain, placenta and erythrocytes. The transporter was initially considered to be a non-insulin-sensitive glucose
transporter, mainly involved in the basal glucose uptake of the cells. However, it has been shown that this transporter is translocated after insulin stimulation of adipocytes (Gould et al. 1989; Zorzano et al. 1989), together with the insulin-responsive glucose transporter (GLUT4) (James et al. 1989), raising the question of the relative contribution of the GLUT1 transporter to the action of insulin on glucose uptake. The GLUT4 glucose transporter gene, which has recently been cloned, appears to be another good candidate gene for NIDDM. It is expressed in adipocytes and muscle cells (Joost and Weber 1989), which are sites of insulin-dependent glucose disposal; a defect in this protein should therefore be considered in the aetiology of NIDDM.

We have previously reported a significant association between a genetic variant at the GLUT1 transporter and NIDDM in three distinct ethnic groups (Li et al. 1988); however, a study in a Chinese American population has failed to confirm this association (Xiang et al. 1989). In another study with four restriction fragment length polymorphisms (RFLPs) in Black Americans, there was no significant association between any of these RFLPs and NIDDM, when the results were corrected for multiple comparisons (Kaku et al. 1990). Further work is required to assess the possible contribution of the GLUT1 (HepG2) transporter gene to the inheritance of NIDDM, and we have therefore studied, in a newly recruited Italian diabetic population, two individual RFLPs at this locus, detected with the restriction enzymes XbaI and StuI (Shows et al. 1987; Li et al. 1989a), and their related haplotypes. We have also examined a KpnI RFLP (Bell et al. 1989) at the muscle/adipocyte GLUT4 transporter.

Materials and methods

A total number of 106 subjects was newly recruited for the study; 48 unrelated Italian Caucasian patients (21 women, 27 men) were recruited from the diabetic clinic of the II Clinica Medica, University of Rome. All conforms to the World Health Organization criteria for non-insulin-dependent diabetes mellitus (WHO 1980). Unrelated Italian Caucasian normal subjects (36 women, 21 men) were selected for the control group on the basis of their fasting blood glucose levels being less than 6 mmol/l, and their having a negative family history for diabetes in their first degree relatives. Not all subjects were genotyped at each RFLP, and the number of observations at each RFLP are indicated in the relevant tables (see Tables 2, 5). The clinical details of the two groups are described in Table 1.

Analysis of RFLPs

DNA was extracted from 10ml whole blood as previously described (Kunkel et al. 1977). Briefly, 8μg DNA was digested with the restriction enzymes XbaI, StuI and KpnI according to the manufacturer's instructions (GIBCO-BRL, Uxbridge, UK). The resulting fragments were separated by electrophoresis in 0.85% agarose gel, and subsequently transferred to Hybond-N filters (Amersham International, Amersham, Bucks) by Southern blotting. The filters were then hybridized with a genomic DNA probe labelled with P32 by the random priming procedure, and the bands were visualized by autoradiography after incubation at -70°C with “Hyperfilm” (Amersham) for 2–10 days. The GLUT1 probe used for hybridization was prepared from the 3.5-kb genomic GLUT1 transporter probe Pela, kindly donated by Dr. M. Mueckler (Washington University, Missouri). This probe was digested with SphI (GIBCO-BRL) to yield a 2.5-kb fragment pea-SphI covering the 5’ region upstream of exon 3 of the GLUT1 gene (Kaku et al. 1990). The GLUT4 cDNA transporter probe used was the 1.7-kb EcoRI insert phJHT-3 (Bell et al. 1989) covering a 1326-bp region encoding for amino acids 1-442, kindly donated by Dr. G. I. Bell (University of Chicago, Illinois).

Statistical analysis

Differences in genotype and allelic frequencies were assessed by Chi-square analysis with Yates’ correction where indicated. The polymorphism information content (PIC) value was calculated as previously described (Botstein et al. 1980).

Linkage disequilibrium between the XbaI and the StuI alleles was assessed by the likelihood ratio test, comparing estimated haplotype frequencies with those expected from the allele frequencies assuming no linkage disequilibrium, and by the disequilibrium statistics Delta (Hill and Robertson 1968) and D' (Lewontin 1984).

When Delta is calculated, complete linkage disequilibrium between two sites is detected when Delta = ± 1, and complete random association when Delta = 0, using nDelta² (n = sample size) as the variable with 1 df to test the null hypothesis (Delta = 0). D' provides an indication of the linkage disequilibrium relative to its theoretical maximum. Linkage disequilibrium between the XbaI and StuI sites was assessed in the whole population studied, and for each group individually.

Comparison of haplotype frequencies was performed by the likelihood ratio test. In the absence of family data, it is not possible unambiguously to haplotype subjects who are heterozygous at both sites; to overcome this problem, we have estimated the frequency of the haplotypes by maximum likelihood from the number of unambiguous haplotypes obtained (Hill 1974), assuming that the genotype frequencies for the double heterozygote class are the same as those computed for the other classes. Comparable results were obtained when the haplotype frequencies were estimated with the method described by Morgan et al. (1990). Control and NIDDM patients haplotypes were estimated separately for each group.

Results

RFLPs at the GLUT1 transporter

Two RFLPs were identified at the GLUT1 transporter with the genomic DNA probe Pela-SphI: one with the restriction enzyme XbaI (Shows et al. 1987), resulting in

Table 1. Clinical details of the Italian Caucasian population studied.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Age (years)</th>
<th>BMI (w/h^2)</th>
<th>Fasting blood glucose (mg/dl)</th>
<th>Duration (years)</th>
<th>Positive FH</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIDDM</td>
<td>48</td>
<td>60.2 ± 11</td>
<td>27.4 ± 3.9</td>
<td>9.4 ± 0.4</td>
<td>10 ± 8</td>
<td>22 (45%)</td>
</tr>
<tr>
<td>Controls</td>
<td>58</td>
<td>53.3 ± 10</td>
<td>25.7 ± 3.1</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation, BMI, weight/height^2