Brief Communications

Lack of Arg972 Polymorphism in the IRS1 Gene in Parakanã Brazilian Indians

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Abstract Several polymorphisms in the insulin receptor substrate-1 (IRS1) gene have been reported in the last years. The most common IRS1 variant, a Gly → Arg substitution at codon 972 (Arg972 IRS1), is more prevalent among subjects who have features of insulin resistance syndrome associated, or not, with type 2 diabetes in European populations. To determine whether the absence of IRS1 polymorphism is a more general characteristic of Paleo-Indian-derived populations, we examined the Arg972 IRS1 polymorphism in Parakanã Indians and found a lack of this polymorphism in the Parakanã population.

Insulin resistance, defined as a subnormal response to a given concentration of insulin, is an important pathophysiological factor in such prevalent diseases as diabetes mellitus, hypertension, obesity, and metabolic syndrome (Cheatham and Kahn 1995). Genetic and acquired factors can profoundly influence insulin sensitivity. Early and intermediate steps in the insulin signaling cascade have been considered candidates for genetic defects that cause insulin resistance.

Insulin exerts its action by binding to its cell-surface transmembrane receptor. This stimulates receptor autophosphorylation and activation of the intrinsic tyrosine kinase activity, which results in tyrosine phosphorylation of several intracellular substrates (Cheatham and Kahn 1995). Over the past decade, a number of these substrates have been characterized. The first and major substrate of the insulin receptor is insulin receptor substrate-1 (IRS-1). After insulin stimulation, the tyrosine phosphorylated IRS-1 serves as multisite docking proteins for various effector molecules possessing src homology 2 (SH2) domains. IRS-1 is ubiquitously expressed in tissues that are responsible for glucose production, glucose uptake, and insulin production (Saad et al. 1992; Rothenberg et al. 1995), and it

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plays a major role in mediating both metabolic and mitogenic effects of insulin (Defronzo 1997).

Several polymorphisms in the IRS1 gene have been reported in the last nine years (Almind et al. 1993; Imai et al. 1994; Hitman et al. 1995; Zhang et al. 1996). The most common IRS1 variant, a Gly → Arg substitution at codon 972 (Arg972 IRS1), is more prevalent among subjects who have features of insulin resistance syndrome associated, or not, with type 2 diabetes in European populations (Hitman et al. 1995; Zhang et al. 1996; Sigal et al. 1996). However, this polymorphism is rare or absent in the Pima Indians of Arizona, a well-defined population with a high prevalence of insulin resistance, suggesting that this polymorphism is not responsible for reduced insulin sensitivity in these Amerindians (Celi et al. 1995). To determine whether the absence of IRS1 polymorphism is a more general characteristic of Amerindian-derived populations, we examined the Arg972 IRS1 polymorphism in Parakanã Indians, an Amazonian Indian population belonging to the Tupi tribe.

Materials and Methods

We searched for the Arg972 polymorphism of the IRS1 gene in 64 Parakanã Indians among whom it was possible to exclude first-degree relationships and in 194 newborns delivered at the University Hospital of Campinas (the control group). The study was approved by the Ethical Committee of the University Hospital.

Genomic DNA was extracted from peripheral blood leukocytes by means of standard techniques using phenol–chloroform. The Parakanã Indians and the control group were genotyped for the Arg972 polymorphism of the IRS1 gene using the polymerase chain reaction (PCR). The forward primer was 5'-CTTCTGTCAGGTGTCCATCC-3', and the reverse primer was 3'-CGATGCACCTGTGGAGCGGT-5' (Almind et al. 1993). The product of amplification (263 bp) was subsequently digested with the restriction enzyme MvaI. The digested products were run in 3.5% agarose gel stained with ethidium bromide, and the digestion patterns were used to determine the presence or absence of codon 972 polymorphism.

The SAS system from Windows (version 6.1) was used for statistical analysis, and the Fisher test was employed. P values less than 0.05 were considered significant.

Results and Discussion

PCR RFLP analysis showed a lack of the Arg972 polymorphism in the IRS1 gene in the Parakanã Indians, whereas in the control group the allele frequency of the mutated allele was 7%, which is statistically significant, as shown in Table 1.
Table 1. Number of Individuals with Arg972 Polymorphism in the IRS1 Gene: Brazilian Control Group and Parakanã Indians

<table>
<thead>
<tr>
<th>IRS1 Genotype</th>
<th>Sample</th>
<th>Gly/Gly</th>
<th>Gly/Arg or Arg/Arg</th>
<th>P Value (Fisher Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal control group</td>
<td>168</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Parakanã Indians</td>
<td>64</td>
<td>0</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

The Amerindians are unlike any other population of similar size or antiquity, in that they have undergone evolution in virtual isolation for 15,000–20,000 years (Gibbons 1993). The Parakanã Indians are derived from the Tupi tribe and first established contact with neo-Brazilians in 1971. These Indians constitute a very small population (about 450 individuals in 1994, when the samples were collected) and are distributed throughout 3 villages in the north of Pará State (northern Brazil). This group is polygamous and favors marriage of males to their sister’s daughters. As a result, the population is quite inbred (Black et al. 1981). Therefore the bottleneck effect, to which this population has been subjected during its evolution, is not counteracted by the broadening of their gene pool through exogamous gene flow.

The Parakanã Indians have a restricted range of polymorphism for several genetic markers, including blood groups, the HLA system, hemoglobin, red cell enzymes, and serum protein phenotypes. Analysis of 13 biallelic systems in the Parakanã Indians shows that they are more distant from the South American Indian founders and from Europeans than 40 other tribes (Black et al. 1981).

The genetics of insulin resistance is the result of a polygenic interaction involving the simultaneous inheritance of a number of widespread mutations. Mutation in genes that encode proteins involved in insulin signal transduction pathways could result in insulin resistance and type 2 diabetes (Pederson 1999). Several research groups have reported IRS1 gene polymorphisms in patients with type 2 diabetes, as shown in Table 2 (Almind et al. 1993; Imai et al. 1994; Hitman et al. 1995; Zhang et al. 1996; Pederson 1999). However, a significant amount of

Table 2. Frequency of Arg972 Polymorphism in the IRS1 Gene: Present Study and Published Studies on Different Ethnic Groups

<table>
<thead>
<tr>
<th>Sample</th>
<th>Frequency in Individuals with Type 2 Diabetes (%)</th>
<th>Frequency in Control Group (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parakanã Indians</td>
<td>Unknown</td>
<td>Absent</td>
</tr>
<tr>
<td>Pima Indians</td>
<td>Rare or absent</td>
<td>Rare or absent</td>
</tr>
<tr>
<td>Finnish</td>
<td>9.8</td>
<td>8.7</td>
</tr>
<tr>
<td>Danish</td>
<td>11.6</td>
<td>4.0</td>
</tr>
<tr>
<td>French</td>
<td>9.9</td>
<td>6.9</td>
</tr>
<tr>
<td>South Indian</td>
<td>10.3</td>
<td>5.3</td>
</tr>
<tr>
<td>Italian</td>
<td>22.6</td>
<td>12.6</td>
</tr>
<tr>
<td>Mexican American</td>
<td>6.5</td>
<td>11</td>
</tr>
<tr>
<td>Japanese</td>
<td>5.0</td>
<td>4.3</td>
</tr>
</tbody>
</table>
these polymorphisms were also found in nondiabetic patients. The analyses of different ethnic groups have identified a substitution at codon 972 of the IRS1 gene with a carrier prevalence of 4–12.6% in nondiabetic individuals and 5–22.6% in individuals with type 2 diabetes. Taken together, the results of the present study and the results described for Pima Indians suggest that the absence of the Arg972 polymorphism in the IRS1 gene is a more general characteristic of the Paleo-Indian-derived population. In addition, we suggest that this mutation could have emerged after the migration of Asians to the Americas or that the Parakanã Indians lost this mutation sometime during their evolution through a combination of founder effects and genetic drift.

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