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Linkage study of the glucagon receptor gene with type 2 diabetes mellitus in Italians

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Abstract

Type 2 diabetes mellitus (T2DM) is a common complex trait disorder. Multiple genome scans have identified different loci in linkage with T2D, including a locus on chromosome 17q24-25. Because the glucagon receptor gene (*GCR*) resides on chromosome 17q25, it might be responsible for the linkage identified in the same region. In a combined French-Sardinian study of *GCR*, there is an association of $Gly^{40}Ser$ mutation with T2DM, confirmed by a UK study but not by others. Our goal was to study this selected region of chromosome 17 in a group of Italian patients with late- and early-onset T2DM by genotyping the microsatellites D17S801, D17S937, and D17S1806 and by performing nonparametric multipoint linkage analysis (Merlin 2000-2002) with allele frequencies calculated from sib-pairs data. We recruited from the center of Italy late-onset sib pairs with T2DM and families with maturity-onset diabetes of the young/early-onset T2DM (N = 503). The linkage analysis at chromosome 17q25 reported no positive lod scores in the total T2D sib pairs, in the late-onset T2D group, and in the early-onset T2D group. Although the study does not show evidence for linkage in this chromosomal region in our Italian cohort, we cannot a priori exclude the possibility of an allelic or genotypic association. Nevertheless, we may conclude that *GCR* does not play a major role in the pathogenesis of T2DM in Italians.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a complex trait disorder characterized by defective insulin secretion and impairment of insulin action in peripheral tissues. The late-onset T2DM form is highly polygenic. The early-onset T2DM involves patients diagnosed before reaching age 40 years and patients with 6 identified autosomal dominant forms of maturity-onset diabetes of the young (MODY). The genetic inheritance of T2DM is supported by a high concordance rate in monozygotic twins, family clustering of disease, and high phenotype diversity in the incidence and prevalence among different ethnic groups. The inheritance model of T2DM is still indefinite and is complicated by the presence of environmental factors and genetic heterogeneity. Multiple genome-wide scans performed in different populations identified different chromosomal loci in linkage with T2DM: NIDDM1 (chromosome 2q, calpain-10 gene), NIDDM2 (chromosome 12q), and several others, including a locus on chromosome 17q24-25 (Botnia region) [1].

The glucagon receptor gene (*GCR*) resides on chromosome 17q25 [2] and therefore might be responsible for the linkage identified in the same region [1]. A combined French and Sardinian study of the *GCR* gene reported an association of the $Gly^{40}Ser$ mutation with T2DM [3], a finding confirmed in a UK study [4] but not in others [5-7]. Glucagon receptor gene mutations may be responsible for high hepatic glucose output in T2D owing to increased glycogenolysis and/or gluconeogenesis, for hyperglucagonemia owing to impairment of endogenous glucagon autofeedback, and for altered insulin secretion owing to glucagon resistance of the β cell. As most studies focused on $Gly^{40}Ser$, we hypothesized that there might be an unknown genetic variation in the *GCR* gene responsible for T2DM and

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that the genetic variant $Gly^{40}Ser$ identified in the French population might as well be a cause for T2DM in the Italian population, which is genetically very close to the French one. Therefore, we performed a selected linkage study in the *GCR* region for T2DM in our Italian cohort to exclude/confirm a role for this gene in the pathogenesis of T2DM.

2. Methods

We recruited from the center of Italy late-onset sib pairs with T2DM and families with MODY/early-onset T2DM (N = 503). All subjects gave written informed consent. The Institutional Review Board of the Massachusetts General Hospital (Boston, Mass) approved the study. Probands were recruited based on the diagnosis of T2DM following the criteria of the National Diabetes Data Group and on the basis of positive family history for T2DM. All patients were descendents of Italian ancestors and lived in the area of Rome. The recruited group consists of 174 sib pairs with T2DM and 29 sib pairs/families with early-onset T2DM/MODY. Among the 29 early-onset T2DM sib pairs/families, 4 have identified mutations in MODY3, 2 in MODY2, and 1 in MODY4 [8-10].

We genotyped patients by using 3 fluorescein-labeled microsatellite markers from the region of the *GCR* gene: D17S801, D17S937, and D17S1806 (103.53, 105.68, 114.41 cM, respectively, on the Marshfield map) with a polymorphism information content of 0.6 or greater (Version 10 Multiplex, Research Genetics, Invitrogen Corp, Carlsbad, Calif). The softwares Genescan and Genotyper (Applied Biosystems, Foster City, Calif) were used. We performed multipoint nonparametric linkage analysis (Merlin 2000-2002/Marshfield map) with allele frequencies calculated from the sib pairs data [11] in the total group of sib pairs/families and separately in the late-onset sib pairs group and in the early-onset sib pairs/families group. The power to detect linkage for a gene with $\lambda_s = 2.0$ and $\theta = 0.00$ between the marker and the disease locus in our sample size of 203 sib pairs/families is approximately 80% and for a gene with $\lambda_s = 2.3$ is approximately 90% [12].

3. Results

The multipoint nonparametric linkage analysis at the chromosomal locus 17q25 reported the maximum lod score 0.00 ($P = .8$, 105.68 cM) in the total T2DM sib pairs, the maximum lod score 0.00 ($P = .7$, 105.68 cM) in the late-onset T2DM group, and the maximum lod score 0.00 ($P = .9$, 105.68 cM) in the early-onset T2DM group. Therefore, we exclude linkage to T2DM in the region containing the *GCR* gene in our Italian T2DM cohort.

4. Conclusions

It is possible that the previously reported association of the *GCR* gene with T2DM in the French-Sardinian populations might be caused by stratification; in fact, there is no proof of association by transmission disequilibrium test in that study. Furthermore, a study in the Sardinian population has shown a geographic variation of $Gly^{40}Ser$ frequency and association of $Gly^{40}Ser$ carriers with a significantly lower increase in plasma glucose in response to glucagon compared with wild-type subjects [13]. Although we found no evidence for linkage in this chromosomal region in our Italian cohort, we cannot a priori exclude the possibility of an allelic or genotypic association. The results of our studies lead us to conclude that the *GCR* likely does not play a major role in the pathogenesis of T2DM in Italians.

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