The Common −866G/A Polymorphism in the Promoter Region of the UCP-2 Gene Is Associated with Reduced Risk of Type 2 Diabetes in Caucasians from Italy

Angela Bulotta, Ornella Ludovico, Angelo Coco, Rosa Di Paola, Alessandro Quattrone, Massimo Carella, Fabio Pellegrini, Sabrina Prudente, and Vincenzo Trischitta

Unit of Endocrinology, Scientific Institute Casa Sollievo della Sofferenza (A.B., O.L., A.C., R.D.P., V.T.), San Giovanni Rotondo, Italy; Unit of Genetics, Scientific Institute Casa Sollievo della Sofferenza (A.Q., M.C.), San Giovanni Rotondo, Italy; Department of Clinical Pharmacology and Epidemiology, Consorzio Mario Negri Sud (F.P.), S. Maria Imbaro, Italy; Casa Sollievo della Sofferenza-Mendel Institute (S.P., V.T.), Rome, Italy; and Department of Clinical Sciences, University La Sapienza (V.T.), Rome, Italy

Uncoupling protein-2 (UCP2) regulates insulin secretion and may play an important role in linking obesity to type 2 diabetes (T2D). Previous studies of the role of the UCP2 promoter −866G/A single nucleotide polymorphisms (SNP) in T2D have given opposite results. We tested the distribution of the −866G/A SNP in 746 T2D patients and 327 healthy unrelated Caucasians from Italy. We also tested for an effect of the P12A variant of the peroxisomal proliferator-activated receptor-γ2 (PPARγ2) gene on diabetes risk given by the UCP2 SNP. Compared with −866G/G carriers, a progressively reduced (P = 0.01) risk of T2D was observed in −866G/A and −866A/A subjects, with the latter showing an approximately 50% risk reduction [odd ratio (OR), 0.51; 95% confidence interval (CI), 0.3–0.8; P = 0.003]. Conversely, the −866G/G genotype was associated with increased risk (OR, 1.31; 95% CI, 1.01–1.71).

Overall, the population risk attributable to the UCP2 −866G/G genotype was about 12%. After stratifying for the PPARγ2 polymorphism, the increased risk conferred by the UCP2 G/G genotype was still evident among P12/P12 homozygous subjects (n = 801; OR, 1.38; 95% CI, 1.04–1.83), but seemed to disappear among the X12/A12 subjects (i.e. P12/A12 heterozygous or A12/A12 homozygous subjects; n = 137; OR, 0.87; 95% CI, 0.40–1.91). Whether this apparent difference is entirely due to the different number of carriers of the two PPARγ2 genotypes is a likely possibility that deserves deeper investigation. In conclusion, in our population, the −866G/A SNP is associated with T2D. Additional studies in larger samples are needed to investigate the possibility of a concomitant effect of modifier genes such as PPARγ2. (J Clin Endocrinol Metab 90: 1176–1180, 2005)

TYPE 2 DIABETES (T2D) and its cardiovascular complications are a tremendous burden on health care systems (1). The combination of insulin resistance and reduced pancreatic β-cell function are pathogenic for T2D (2). Both defects are likely to be under polygenic control, with several genes simultaneously involved (3).

Uncoupling protein 2 (UCP2) belongs to the superfamily of the mitochondrial transporter proteins and is highly expressed in adipose tissue and pancreatic islets (4). By uncoupling β-cell metabolism from ATP generation, UCP2 has been reported to negatively regulate glucose-stimulated insulin secretion (5). UCP2 is, in fact, overexpressed in islets of ob/ob mice, a model of obesity-induced diabetes (6); conversely, when knocking out the UCP2 gene, these mice may be partially rescued from diabetes as a consequence of increased insulin secretion (6). At variance, other studies have reported that in a different model of obesity-induced T2D (i.e. Zucker diabetic fat rats) UCP2 overexpression may favor β-cell function and eventually improve glycemic control (7). Although suggesting an opposite effect, both reports (6, 7) clearly indicate that UCP2 may play an important role in linking obesity to β-cell dysfunction and T2D.

In the Goto-Kazizaki rat, the UCP2 gene is linked to D1Wox38 marker (8) (at 87.9 cM of the rat chromosome 1, www.well.ox.ac.uk/rat_mapping_resources/), which is located in a region where a relevant number of linkage peaks for glucose intolerance and adiposity have been identified (8). In humans, the 11q13 chromosomal region (which contains the UCP2 gene) is weakly linked to T2D in Finns (9), but not in other populations (10). In Finns, by a genome scan performed at an average resolution of 8 cM, the most relevant logarithm of the odds (LOD) score peak in 11q13, lies about 8 cM from the UCP2 gene locus (9).

Taken together, all of these data indicate that the UCP2 gene is an excellent candidate for T2D. A functional −866 G/A single nucleotide polymorphism (SNP) has been described in the UCP2 promoter (11). The −866 A allele has been reported to increase UCP2 transcriptional activity in transfected cultured cells (12); however, data in human tissues have been conflicting, reporting either increased (12) or decreased (13) UCP2 mRNA levels to be associated with the −866 A allele. Finally, despite the fact that the −866A allele is associated with reduced insulin secretion in nondiabetic individuals (14), its role in T2D has been contradictory, with
A allele carriers reported to be at increased (12) or reduced (13) risk. According to the polygenic model proposed for T2D (3), this latter discrepancy might be partly due to the concomitant and confounding effects of modifier genes. Among the genetic variants with a broad impact on the risk of T2D is the highly prevalent P12A polymorphism of the peroxisomal proliferator-activated receptor-γ2 (PPARγ2) gene that encodes for a transcription factor involved in the regulation of adipocyte differentiation and intracellular insulin signalling (15–17). The common PPARγ2 P12A variant is associated with the risk of T2D (15, 18). Interestingly, PPARγ2 modulates UCP2 expression (19, 20). In addition, both agonists and antagonists of the PPARγ effector modulate insulin secretion (20–23). Together these data (19–23) make the possibility of a combined effect of the two genes on the risk of T2D a likely one.

In the present study we evaluated the association of the common −866G/A SNP of the UCP2 gene with T2D in a Caucasian population from Gargano (east coast of central Italy). We also tested for an effect of the P12A variant of the PPARγ2 gene on the diabetes risk conveyed by the UCP2 SNP.

Subjects and Methods

Experimental subjects

Both cases and controls were of Caucasian origin and resident of the same region (i.e. Gargano and surrounding areas, east coast of central Italy). Cases were 746 (382 men and 364 women) patients with T2D consecutively recruited from the Endocrine Unit at Scientific Institute CSS (San Giovanni Rotondo, Italy) who met the following criteria: 1) diabetes diagnosed after 30 yr, 2) insulin treatment not required for at least 2 yr after diabetes diagnosis, and 3) absence of clinically evident autoimmune disease. The clinical features of patients studied are shown in Table 1. To quantify the risk of recruiting late-onset type 1 diabetic patients, anti-glutamic acid decarboxylase antibodies were determined in 200 randomly selected patients: only three of 200 patients were anti-glutamic acid decarboxylase positive (≥0.9 U/ml), indicating that the risk of misdiagnosis is trivial in our sample. The control group consisted of 327 (116 men and 211 women) unrelated subjects with the same age range (35–76 yr) as cases who were recruited in the same area as part of an ongoing study of the genetics of insulin resistance. Selection criteria were as follows: fasting plasma glucose less than 6.1 mmol/liter, no medications known to affect glucose and lipid metabolism, and absence of systemic diseases. Clinical features of control subjects are shown in Table 1.

TABLE 1. Clinical features of TD2 patients

<table>
<thead>
<tr>
<th>No.</th>
<th>746</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr) (range)</td>
<td>60.4 ± 8.7 (35–76)</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>382/364</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.9 ± 5.6</td>
</tr>
<tr>
<td>Duration of disease (yr)</td>
<td>10.2 ± 8.6</td>
</tr>
<tr>
<td>Hypertension [%]</td>
<td>8.6 ± 2.0</td>
</tr>
<tr>
<td>Dyslipidemia [%]</td>
<td>616 (83)</td>
</tr>
<tr>
<td>Antidiabetic treatment [no. (%)]</td>
<td>639 (86)</td>
</tr>
<tr>
<td>None</td>
<td>96 (12.9)</td>
</tr>
<tr>
<td>OHA</td>
<td>350 (46.9)</td>
</tr>
<tr>
<td>Insulin (±OHA)</td>
<td>300 (40.2)</td>
</tr>
</tbody>
</table>

Data are the mean ± sd (and range, when indicated, in parentheses). Hypertension, ≥130/85 mm Hg, or currently receiving antihypertensive treatment. Dyslipidemia: total cholesterol, ≥200 mg/dl; high-density lipoprotein cholesterol, ≤40 mg/dl in males and 50 mg/dl in females; triglycerides, >150 mg/dl or currently receiving lipid-lowering treatment. Treatment: none; diet only; OHA, oral hypoglycemic agents. HbA1c, Hemoglobin A1c.

Genotyping

Genomic DNA was extracted from peripheral blood according to standard procedures. The −866G/A polymorphism in the UCP2 gene was determined by digesting PCR products with restriction enzyme MluI (Invitrogen Life Technologies, Inc., San Diego, CA) as previously described (24). The PPARγ2 P12A polymorphism was screened by dideoxy single-base extension of an unlabeled primer, according to the manufacturer instructions (ABI PRISM SNaPshot Multiplex Kit 4323151, Applied Biosystems, Foster City, CA) as previously described (25).

Statistical analyses

Data are expressed as the mean ± sd. Categorical variables and continuous ones were, respectively, compared by χ² test and one-way ANOVA. Logistic regression analysis was used to assess the extent to which the −866G/A polymorphism or the P12A variant of the PPARγ2 gene was associated with T2D. The same analysis was used to model the effect of each single polymorphism and their interaction on the risk of T2D. P values for the combined effect of the two SNPs on diabetes risk were also assessed through permutation analysis (26) of 1000 random replicates using an SAS macro language routine written by one of the authors.

As expected, although recruited within the same age range (i.e. 35–76 yr), controls were younger and leaner than T2D patients; therefore, when indicated, logistic regression models are adjusted for age and body mass index (BMI).

All analyses were performed using the SPSS software program version 12.0 for Windows (SPSS, Inc., Chicago, IL), and SAS (SAS release 8.2, 1999–2001, SAS Institute, Inc., Cary, NC).

Results

The proportion of individuals carrying the three possible genotypes (−866G/G, −866G/A, and −866A/A) was significantly different between controls and T2D patients, with a trend toward a progressively reduced risk of T2D from heterozygous to homozygous subjects for the −866A allele (Table 3). Overall, a significant association of the UCP2 −866G/A genotype with T2D risk was observed [odd ratio (OR), 0.69; 95% confidence interval (CI), 95%
TABLE 3. Distribution of the UCP2-866 G/A polymorphism in T2D patients and controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>T2D</th>
<th>Controls</th>
<th>(P^a)</th>
<th>OR (95% CI) Adjusted(^b) OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−866 G/G</td>
<td>374</td>
<td>142</td>
<td>0.01</td>
<td>1</td>
</tr>
<tr>
<td>−866 G/A</td>
<td>317</td>
<td>144</td>
<td>0.84</td>
<td>(0.6–1.1)</td>
</tr>
<tr>
<td>−866 A/A</td>
<td>55</td>
<td>41</td>
<td>0.51</td>
<td>(0.3–0.8)</td>
</tr>
</tbody>
</table>

\(^a\) By \(\chi^2\) analysis (2 \times 3 table) between T2D and controls.
\(^b\) Adjusted for age, gender, and BMI.

0.51–0.93; \(P < 0.015\); age, gender, and BMI adjusted]. Accordingly, the resulting population risk attributable to the UCP2 −866G/G genotype (i.e., the excess rate of disease in the studied population attributable to this genotype) was approximately 12%.

The modulation of T2D risk is believed to be polygenic, with several genes simultaneously involved (3). A consistent impact on the risk of T2D is given by the highly prevalent PPARγ2 P12A polymorphism (13, 16). We then looked for the combined effect of UCP2 and PPARγ2 genes on the risk of T2D in individuals for whom both genotypes were available (i.e., 1038 of the 1073 study subjects). Subjects carrying the −866G/G had an increased risk of T2D compared with subjects carrying the −866A allele (Table 4, left panel). Although no effect of the PPARγ2 P12A genotype per se on the risk of T2D was observed, at least with the present sample size, in our population (OR, 0.76; 95% CI, 0.42–1.37; \(P = 0.36\)), the risk for −866G/G subjects was increased in PPARγ2 P12/12 homozygous (Table 4, middle panel), but not in PPARγ2 X12/A12 (i.e., either P12/A12 or A12/A12) subjects (Table 4, right panel). A similar negative finding among PPARγ2 X12/A12 subjects was also obtained by permutation analysis (\(P = 0.83\)). Overall, with the present sample size, the \(P\) value for gene–gene interaction, as tested by regression logistic analysis (see Subjects and Methods), is not statistically significant (\(P = 0.16\)).

No differences were observed across the three genotype groups as far as glucose, insulin, and lipid profile was concerned in both controls and T2D patients (data not shown). Also, no difference in BMI values was observed across the three genotype groups in controls or T2D patients, making it unlikely that in our population the protective effect of the UCP2 −866G/G allele on the risk of T2D may act through changes in adiposity. The UCP2 −866A allele has been variably associated with both increased (12) and decreased (13) UCP2 mRNA levels in human tissues. Similarly, UCP2 overexpression has been reported to either increase (7) or decrease (5, 6) insulin secretion in rodent pancreatic islets. It is therefore possible that the protective effect on the risk of T2D of the UCP2 −866A allele may act through the modulation of UCP2 expression in pancreatic islets. As an alternative explanation, the UCP2 −866G/A SNP might have no effect per se on the risk of T2D; rather, it may represent only a marker in linkage disequilibrium with another, causative, genetic variant(s). In fact, as inferred from HapMap genotype data from Caucasian populations, the −866G/A SNP lies in a 17-kb block covering the entire gene and its flanking sequences. In this block, the linkage disequilibrium remains high from 5' upstream SNPs such as rs591758 (\(D' = 1.0\); \(r^2 = 0.89\)) to more distal SNPs such as (Ala55Val)rs660339 (\(D' = 0.89\).
TABLE 4. Distribution of UCP2 −866 G/A and PPARγ P12A polymorphisms in T2D patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Whole population</th>
<th>PPARγ P12P</th>
<th>PPAR-X12A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T2D [no. (%)]</td>
<td>Control [no. (%)]</td>
<td>T2D [no. (%)]</td>
</tr>
<tr>
<td>UCP2 G/G</td>
<td>358 (50.3)</td>
<td>142 (43.6)</td>
<td>308 (50.7)</td>
</tr>
<tr>
<td>UCP2 X/A</td>
<td>354 (49.7)</td>
<td>184 (56.4)</td>
<td>300 (49.3)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.31 (1.01–1.71)</td>
<td>1.38 (1.04–1.83)</td>
<td>0.87 (0.40–1.91)</td>
</tr>
<tr>
<td>P</td>
<td>0.04</td>
<td>0.025</td>
<td>0.73</td>
</tr>
</tbody>
</table>

PPARγ X12A, Either P12A (n = 134) or A12A (n = 3) individuals; UCP2 X/A, either G/A (n = 444) or A/A (n = 94) individuals.

= 1.0; \( r^2 = 0.83 \) and even farther beyond (data not shown). Having such a central position and such a high \( r^2 \) values, in comparison with other SNPs (rs591758 vs. rs660339 has \( D' = 1.0 \) and \( r^2 = 0.73 \)), we believe that −866G/A is a very informative SNP for the entire UCP2 gene.

Whatever the mechanisms underlying the reported association, our results are in agreement with previous findings showing a protective effect on the risk of T2D for the A allele (13), but are the opposite of those obtained among obese individuals (12). An intriguing possibility for partly explaining some of these inconsistencies is that the contribution of the UCP2 −866G/A SNP to T2D risk may be different in different samples depending on different genetic background. By reanalyzing our data according to the PPARγ2 P12A genotype, the risk of T2D for subjects carrying the UCP2 −866G/G genotype was significantly increased in the PPARγ2 P12/P12 homozygous subjects, whereas no effect was observed in individuals carrying the PPARγ2 A12 variant. Although no significant interaction (\( P = 0.16 \), by logistic regression analysis) was obtained with our present sample size, and we, therefore, cannot conclude about gene-gene interaction, our present data may serve the function of hypothesis-generating to be tested in larger samples. In fact, this study clearly underlines the problem of trying to investigate interaction between genes whose genotype frequencies are rather rare and indicate the need for very large samples to be recruited for this purpose. Although the final net effect on insulin secretion has been controversial (20–23), several studies have clearly demonstrated that PPARγ2 induces UCP2 up-regulation in rat pancreatic islets (19, 20). In humans, two putative PPARγ2 response elements with a DR1 half-site (AGGTCA), one of which located in the close proximity to the −866G/A SNP, have been identified, thus suggesting a potential regulation of UCP2 transcription by PPARγ (28). All of these data make the possibility of a combined effect of the two genes on the risk of T2D a likely one. It is worth noting that while our manuscript was in preparation, a significant interaction on the risk of T2D was reported among Asians between two different polymorphisms of the same genes (i.e. the UCP2 Ala\(^{53}\)Val variant and the silent C→T substitution in the exon 6 of PPARγ2 gene (29).

In case-control studies, population stratification bias may occur and may cause false positive results (30). Because the association between this SNP and T2D has been reported in other populations (12, 13), we believe this risk is minimized in our study. In addition, the distribution of polymorphic microsatellite loci in subgroups of randomly selected T2D patients and controls suggests the lack of differential genetic admixture between these two groups (data not shown). Although, as indicated by very recent data (31), the number of individuals studied for this analysis may be insufficient to draw firm conclusions, this finding is compatible with the lack of a population stratification bias in our study.

In conclusion, in the Gargano Caucasian population, the −866 A allele in the promoter region of the UCP2 gene is associated with a reduced risk of T2D. Conversely, an approximately 12% population risk of T2D was attributable to the −866G/G genotype. Additional studies in larger samples also investigating the concomitant role of other potential diabetogens are likely to help in understanding the role of the UCP2 gene in polygenic control of T2D.

Acknowledgments

We thank C. Cistermino (B.S.) and G. Fini (B.S.) for technical support. We also thank Dr. G. Sesti (University of Catanzaro, Catanzaro, Italy) for kindly providing us with control sequence samples, and Dr. Vittorio Tassi (CIS Institute, San Giovanni Rotondo, Italy) for helpful discussion.

Received June 7, 2004. Accepted November 10, 2004.

Address all correspondence and requests for reprints to: Dr. Angela Bulotta, Unit of Endocrinology, Scientific Institute Casa Sollievo della Sofferenza, Poliambulatorio Giovanni Paolo II, Viale Padre Pio, 71013 San Giovanni Rotondo (FG), Italy. E-mail: a.bulotta@operapadrepio.it. This work was supported by Italian Ministry of Health Grants Ricerca Finalizzata 2002 (to V.T.) and Ricerca Corrente 2003 (to R.D.P.).

References


