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The −8503 G/A Polymorphism of the Adiponectin Receptor 1 Gene Is Associated With Insulin Sensitivity Dependent on Adiposity

Adiponectin has beneficial effects on insulin sensitivity. Unexpectedly, adiponectin knockout mice exhibit no or only mild insulin resistance. Nevertheless, under a high-fat/high-carbohydrate diet, severe insulin resistance was induced in those animals (1). Consistent with this, recent evidence (2) suggests that the relationship of adiponectin with insulin sensitivity is stronger with increasing adiposity. In addition, a haplotype in the adiponectin gene was associated with type 2 diabetes only in obese and morbidly obese subjects but not in lean subjects (3).

Single nucleotide polymorphisms (SNPs) of the genes encoding adiponectin receptor (ADIPOR) 1 and 2 were associated with type 2 diabetes (4) or prediabetes phenotypes (5) in some but not in all (6) studies. We found that the −8503 *G/A* SNP of the *ADIPOR1* gene was associated with insulin sensitivity (7). In a very recent study (6) in rather lean subjects with a mean BMI of 21 kg/m^2, no associations with insulin sensitivity were found. In our study (7), subjects were more obese (BMI 26 kg/m^2). This new information lead us to investigate whether the association of the −8503 *G/A* SNP of *ADIPOR1* with insulin sensitivity is modulated by adiposity. If this was the case, then this may partly explain the inconsistent results regarding SNPs of *ADIPOR1* and 2.

Recently reported data (7) from 502 nondiabetic Caucasians were analyzed. Insulin sensitivity was estimated from an oral glucose tolerance test and determined during a euglycemic-hyperinsulinemic clamp ($n = 299$). Subjects were divided into two groups by the median percentage of body fat (PFAT). In the more obese group ($n = 250$, PFAT 27–55%), carriers of the −8503 *A* allele had lower insulin sensitivity estimated from the oral glucose tolerance test using the formula proposed by Matsuda and DeFronzo (8) (*G/G*, *G/A*, and *A/A*: 14.4 ± 0.8, 11.9 ± 0.9, and 9.2 ± 1.7 arbitrary units, respectively, $P = 0.003$, ANOVA) and determined during the clamp (0.07 ± 0.005, 0.06 ± 0.005, and 0.04 ± 0.01 $\mu mol \cdot kg^{-1} \cdot min^{-1} \cdot pmol/l^{-1}$, respectively, $P = 0.007$) compared with homozygous carriers of the G allele, independent of age, sex, and PFAT. In contrast, in the lean group ($n = 252$, PFAT 7–26%), no significant relationships were found (oral glucose tolerance test: 24.6 ± 1.0, 27.2 ± 1.1, and 23.0 ± 2.4, respectively, $P = 0.60$; clamp: 0.13 ± 0.006, 0.13 ± 0.007, and 0.12 ± 0.016, respectively, $P = 0.84$).

In summary, we show that the A allele of the −8503 *G/A* SNP of the *ADIPOR1* gene is associated with less insulin sensitivity only in more obese but not in lean individuals. This finding may be important for further studies on the relationships of genetic variants of *ADIPOR1* and possibly of *ADIPOR2* with metabolism.

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Evaluation of a Diagnostic Algorithm for Hereditary Hemochromatosis in 3,500 Patients With Diabetes

Hereditary hemochromatosis may lead to hepatic cirrhosis, cardiomyopathy, diabetes, arthritis, and impotence (1,2). In the Caucasian population, *HFE* gene mutations (C282Y and H63D) are present in the majority of patients demonstrating phenotypic expression (3–6). Conversely, the clinical penetrance in mutation carriers is low (7).

In the precirrhotic stage, ~20% of hemochromatosic patients demonstrate hyperglycemia, with the prevalence increasing to >70% in the presence of liver cirrhosis (8). Two mechanisms contribute to the development of hyperglycemia and diabetes. Liver iron overload leads to insulin resistance, and the pancreatic β-cell iron accumulation results in cell damage and diminished insulin secretion (1). The

prevalence of genotypic and phenotypic hemochromatosis is higher in diabetic versus nondiabetic populations (9–11).

In an attempt to distinguish patients with hemochromatosis-associated secondary diabetes from other forms of diabetes, we applied a screening program to diabetic patients aged ≥40 years. The present report summarizes our first 3 years' experience.

From the year 2000, in-hospital patients with diabetes have been invited to participate in the screening program (12). The initial phenotypic screening consisted of serum iron and transferrin analyses and calculation of the transferrin saturation. Elevated transferrin saturation defined as >45% was confirmed by an independent second analysis, which included determinations of serum ferritin and liver enzymes (alanine aminotransferase, aspartate aminotransferase, and γ-glutamyl transpeptidase). Patients with a phenotype indicative of iron overload were genotyped for the presence of aberrant *HFE* gene variants (C282Y, H63D, and S65C) (13,14).

Among 3,500 diabetic patients, elevated transferrin saturation at two occasions was observed in 22 male (aged 52.9 ± 11.6 years) and in 12 female (aged 49.1 ± 14.0 years) subjects, resulting in an iron overload phenotype in 0.97% of the patients. Mutations in the *HFE* gene were identified in seven patients (20.6%), with five of them demonstrating homozygosity for the variant allele at position 282. In addition, one case of compound heterozygosity was documented (C282Y/H63D). The new splice site *HFE* gene mutation (IVS5 + 1 G/A) in one patient of Vietnamese origin has been reported separately (15). Serum ferritin concentration was increased in five of seven patients, whereas pathological liver enzyme activities were noted in four patients. The remaining 27 patients were characterized by the absence of the three most common *HFE* gene mutations. Among them, 13 patients demonstrated elevated serum ferritin concentrations and 12 patients pathological liver enzyme activities.

Phenotype- and genotype-based screening programs have variably been used to investigate the relationship between iron overload, hemochromatosis, and its clinical manifestations including diabetes (16). Using transferrin saturation as a clinically useful initial screening parameter (12,16) to detect iron overload, we obtained evidence for iron overload in only 1% of a large number of diabetic patients. Approximately half of these patients demonstrated liver damage as reflected by increased liver enzyme activities. Genotyping for the most common *HFE* gene mutations revealed hereditary hemochromatosis in only one in seven of these patients, equal to 0.2% of the overall diabetic population investigated in the present study.

The screening program can be performed at a reasonable price and is considered efficient in detecting iron overload in diabetic patients. Direct laboratory costs amount to $2.50 U.S. per patient for the laboratory screening profile (transferrin saturation and liver enzymes) and $5.00 U.S. for the analysis of serum ferritin. The genotyping approach (*HFE* C282Y, H63D, and S65C) results in $40.00 U.S. of additional costs in selected patients. The cost-effectiveness of screening programs for hereditary hemochromatosis was previously assessed. A study (17) of the cost-effectiveness of a population screening approach concluded that screening was an effective strategy for asymptomatic subjects if the prevalence of hemochromatosis was at least 0.3%, accompanied by a probability of disease manifestation >0.4 and test costs <$12.00 U.S. In another study (18), the cost-effectiveness of genetic testing as a screening method was evaluated using a decision model. The model estimated that initial genetic testing would be cost-effective if the cost per test was <$28.00 U.S. Based on the assumption that diabetes is the manifestation of hereditary hemochromatosis, we conclude that a stepwise laboratory diagnostic approach is a cost-effective method for the screening of hereditary hemochromatosis among diabetic patients. In hemochromatosic patients with hyperglycemia/diabetes, it may open the possibility to treat iron accumulation with the aim to prevent further damage to the liver and pancreatic β-cells, which in turn may be expected to contribute to preservation of the remaining insulin secretion, which even at low concentrations may delay the development of diabetes complications (19). Interventional trials should be performed to test the clinical outcome of a combined screening/treatment approach.

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On the Weighted-Average Relationship Between Plasma Glucose and HbA_{1c}

Tahara and colleagues (1–3) have reported results of experimental investigations concluding a relationship between plasma glucose level and HbA_{1c} (A1C), defined as

$$H(t) = K\int_0^t W(t-\zeta)G(\zeta)d\zeta \qquad \text{(Eq. 1)}$$

Their expression for $W(s)$ is

$$W(s) = \begin{cases} 2(T-s)/T^2, & 0 \le s \le T \\ 0, & s > T \end{cases} \qquad \text{(Eq. 2)}$$

The experimental mean fasting plasma glucose (MFPG) results reported in ref. 1 were analytically modeled and then used to determine the corresponding mean A1C curve (Eq. 1). Excellent correlation was obtained between experimental mean A1C and analytical A1C curves. The specific curve for MFPG is

$$G(t) = G_s + G_d \exp(-\gamma t) \qquad \text{(Eq. 3)}$$

where γ is constant in time but nonetheless adjustable to designate different decay rates. The MFPG curve reported in ref. 1 is found from Eq. 3 for $G_s = 6.6$, $G_d = 6.2$, and $\gamma = 1$. The related expression for A1C derived from Eq.1 is

$$\frac{H(t)}{2K} = G_s\left[\left(\frac{t}{T}\right) - 0.5\left(\frac{t}{T}\right)^2\right] + \left(\frac{G_d}{\gamma T}\right)\left(1-\frac{t}{T}\right)\left[1-\exp\left(-\gamma t\right)\right] + \frac{G_d}{\gamma^2 T^2}\left[1-\left(1+\gamma t\right)\exp\left(-\gamma t\right)\right] \qquad \text{(Eq. 4)}$$

Values of $H(t)$ versus t obtained using Eq. 4, with $K = 0.75$, $T = 17$ weeks, and $\gamma = 1$ were subtracted from the mean of patient-admission A1C values to approximate the mean A1C curve reported in ref. 1.

Adjusting γ to designate different decay rates, as would be the case for patients using only diet and exercise to control their plasma glucose levels, it is evident from Eq. 3 that as γ decreases from (say) $\gamma = 1$ to $\gamma = 0.1$, e.g., $\gamma = 0.8, 0.4, 0.2, 0.1$, a family of $G(t)$ curves will be generated whose corresponding decay is slower. Given that 50% of the GHb level at any time is determined by the plasma glucose level during the preceding 1-month period, 25% by the plasma glucose level during the 1-month period before that, and the remaining 25% by the plasma glucose level during the 2-month period before the first 2 months (1–3), it follows that if the plasma glucose curve decays, the corresponding A1C curve will also decay. Results reported in ref. 1 confirm this, as do results from a study by Rohlfing et al. (4), where an algebraic relationship between FPG and A1C is reported.

However, the γ curves of A1C determined from Eq. 4 display a faster decay as γ decreases. This inverted decay suggests the weighted-average relationship between plasma glucose and A1C reported in ref. 1 is questionable. As functions of γ, both plasma glucose and A1C curves should decay faster as γ increases and slower as γ decreases, not one slower and the other faster (or vice versa).

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On the Weighted-Average Relationship Between Plasma Glucose and HbA_{1c}

Response to Treviño

Dr. Treviño (1) has derived a mathematical formula for HbA_{1c} (A1C) change in response to exponential plasma glucose decay. His analysis has shown a faster decay of A1C associated