

unct in the management of diabetic macular edema. *Am J Ophthalmol* 137:675–682, 2004

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/m², no associations with insulin sensitivity were found. In our study (7), subjects were more obese (BMI 26 kg/m²). 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 $0.04 \pm 0.01 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \text{pmol/l}^{-1}$, 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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References

1. Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, Furuyama N, Kondo H, Takahashi M, Arita Y, Komuro R, Ouchi N, Kihara S, Tochino Y, Okutomi K, Horie M, Takeda S, Aoyama T, Funahashi T, Matsuzawa Y: Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med* 8:731–737, 2002
2. Martin LJ, Woo JG, Daniels SR, Goodman E, Dolan LM: The relationships of adiponectin with insulin and lipids are strengthened with increasing adiposity. *J Clin Endocrinol Metab* 90:4255–4259, 2005
3. Vasseur F, Helbecque N, Lobbens S, Vasseur-Delannoy V, Dina C, Clement K, Boutin P, Kadowaki T, Scherer PE, Froguel P: Hypoadiponectinaemia and high risk

of type 2 diabetes are associated with adiponectin-encoding (ACDC) gene promoter variants in morbid obesity: evidence for a role of ACDC in diabetes. *Diabetologia* 48:892–899, 2005

4. Dancott CM, Ott SH, Pollin TI, Reinhart LJ, Wang J, O'Connell JR, Mitchell BD, Shuldiner AR: Genetic variation in adiponectin receptor 1 and adiponectin receptor 2 is associated with type 2 diabetes in the Old Order Amish. *Diabetes* 54:2245–2250, 2005
5. Wang H, Zhang H, Jia Y, Zhang Z, Craig R, Wang X, Elbein SC: Adiponectin receptor 1 gene (ADIPOR1) as a candidate for type 2 diabetes and insulin resistance. *Diabetes* 53:2132–2136, 2004
6. Hara K, Horikoshi M, Kitazato H, Yamachi T, Ito C, Noda M, Ohashi J, Froguel P, Tokunaga K, Nagai R, Kadowaki T: Absence of an association between the polymorphisms in the genes encoding adiponectin receptors and type 2 diabetes. *Diabetologia* 48:1307–1314, 2005
7. Stefan N, Machicao F, Staiger H, Machann J, Schick F, Tschirner O, Spieth C, Weigert C, Fritsche A, Stumvoll M, Haring HU: Polymorphisms in the gene encoding adiponectin receptor 1 are associated with insulin resistance and high liver fat. *Diabetologia* 48:2282–2291, 2005
8. Matsuda M, DeFronzo R: Insulin sensitivity indices obtained from oral glucose tolerance testing. *Diabetes Care* 22:1462–1470, 1999

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 hemochromatotic 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 pa-

tients. 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 hemochromatotic 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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References

- Bacon BR: Hemochromatosis: diagnosis and management. *Gastroenterology* 120: 718–725, 2001
- Barton JC, Edwards CQ (Eds.): *Hemochromatosis: Genetics, Pathophysiology, Diagnosis, and Treatment*. Cambridge, U.K., Cambridge University Press, 2000
- Gottschalk R, Seidl C, Löffler T, Seifried E, Hoelzer D, Kaltwasser JP: *HFE* codon 63/282 (H63D/C282Y) dimorphism in German patients with genetic hemochromatosis. *Tissue Antigens* 51:270–275, 1998
- Bacon BR, Powell LW, Adams PC, Kresina TF, Hoofnagle JH: Molecular medicine and hemochromatosis: at the crossroads. *Gastroenterology* 116:193–207, 1999
- Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, Dormishian F, Domingo R Jr, Ellis MC, Fullan A, Hinton LM, Jones NL, Kimmel BE, Kronmal GS, Lauer P, Lee VK, Loeb DB, Mapa FA, McClelland E, Meyer NC, Mintier GA, Moeller N, Moore T, Morikang E, Prass CE, Quintana L, Starnes SM, Schatzman RC, Brunke KJ, Drayna DT, Risch NJ, Bacon BR, Wolff RK: A novel MHC class I-like gene is mutated in patients with hereditary. *Nat Genet* 13:399–408, 1996
- Mura C, Nousbaum JB, Verger P, Moalic MT, Raguene O, Mercier AY, Ferec C: Phenotype-genotype correlation in haemochromatosis subjects. *Hum Genet* 101: 271–276, 1997
- Bradley LA, Haddow JE, Palomaki GE: Population screening for haemochromatosis: a unifying analysis for published intervention trials. *J Med Screen* 3:178–184, 1996
- Strohmeier G, Niederau C: Diabetes mellitus and hemochromatosis. In *Hemochromatosis: Genetics, Pathophysiology, diagnosis, and Treatment*. Barton JC, Edwards CQ, Eds. Cambridge, U.K., Cambridge University Press, 2000, p. 268–277
- Conte D, Manachino D, Colli A, Guala A, Aimo G, Andreoletti M, Corsetti M, Fraquelli M: Prevalence of genetic hemochromatosis in a cohort of Italian patients with diabetes mellitus. *Ann Intern Med* 128:370–373, 1998
- O'Brien T, Barrett B, Murray DM, O'Sullivan DJ: Usefulness of biochemical screening of diabetes patients for hemo-

- chromatosis. *Diabetes Care* 13:532–534, 1990
11. Singh BM, Grunewald RA, Press M, Muller BR, Wise PH: Prevalence of amongst patients with diabetes mellitus. *Diabetes Med* 9:730–731, 1992
 12. Powell LW, George DK, McDonnell SM, Kowdley KV: Diagnosis of hemochromatosis. *Ann Intern Med* 129:925–931, 1998
 13. Lynas C: A cheaper and more rapid polymerase chain reaction-restriction fragment length polymorphism method for the detection of the HLA-H gene mutations occurring in hereditary hemochromatosis. *Blood* 90:4235–4236, 1997
 14. Mura C, Raguene O, Ferec C: HFE mutations analysis in 711 hemochromatosis probands: evidence for S65C implication in mild form of hemochromatosis. *Blood* 93:2502–2505, 1999
 15. Steiner M, Ocran K, Genschel J, Meier P, Gerl H, Ventz M, Schneider ML, Buttner C, Wadowska K, Kerner W, Schuff-Werner P, Lochs H, Schmidt H: A homozygous HFE gene splice site mutation (IVS5+1 G/A) in a hereditary hemochromatosis patient of Vietnamese origin. *Gastroenterology* 122:789–795, 2002
 16. Adams PC, Reboussin DM, Barton JC, McLaren CE, Eckfeldt JH, McLaren GD, Dawkins FW, Acton RT, Harris EL, Gordeuk VR, Leiendecker-Foster C, Speechley M, Snively BM, Holup JL, Thomson E, Sholinsky P, the Hemochromatosis and Iron Overload Screening (HEIRS) Study Research Investigators: Hemochromatosis and iron-overload screening in a racially diverse population. *N Engl J Med* 352:1769–1778, 2005
 17. Phatak PD, Guzman G, Woll JE, Robeson A, Phelps CE: Cost-effectiveness of screening for hereditary hemochromatosis. *Arch Intern Med* 154:769–776, 1994
 18. Adams PC, Valberg LS: Screening blood donors for hereditary hemochromatosis: decision analysis model comparing genotyping to phenotyping. *Am J Gastroenterol* 94:1593–1600, 1999 (see comments)
 19. Madsbad S: Prevalence of residual B-cell function and its metabolic consequences in type 1 (insulin-dependent) diabetes. *Diabetologia* 24:141–147, 1983

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

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

Their expression for W(s) is

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

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

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

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

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

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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References

1. Tahara Y, Shima K: Kinetics of A1C, glycated albumin, and fructosamine and analysis of their weight functions against preceding plasma glucose level. *Diabetes Care* 18:440–447, 1995
2. Tahara Y, Shima K: The response of GHb to stepwise plasma glucose change over time in diabetic patients (Letter). *Diabetes Care* 16:1313–1314, 1993
3. Shi K, Tahara Y, Noma Y, Yasukawa K, Shima K: The response of glycated albumin to blood glucose change in the circulation in streptozotocin-diabetic rats: comparison of theoretical values with experimental data. *Diabetes Res Clin Care* 17:153–160, 1992
4. Rohlfing CL, Wiedmeyer HM, Little RR, England JD, Tennill A, Goldstein DE: Defining the relationship between plasma glucose and HbA_{1c}: analysis of glucose profiles and HbA_{1c} in the Diabetes Control and Complications Trial. *Diabetes Care* 25:275–278, 2002

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