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ORIGINAL ARTICLE

Adiponectin polymorphisms, adiposity and insulin metabolism: HERITAGE family study and Oulu diabetic study

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Abstract

Aims/hypothesis. Adiponectin is an adipocytokine with lowered blood levels in obesity and Type 2 diabetes mellitus. We sought to define the specific effects of different alleles of the gene encoding adiponectin.

Methods. We studied the associations of adiponectin gene sequence variations with body fat distribution and insulin indices in 503 White and 276 Black subjects of the HERITAGE Family Study cohort and subjects from a Finnish population.

Results. The *His111 allele* frequency of the *Tyr111His polymorphism* in Finnish Type 2 diabetic subjects (n=254) was higher (5.1%) than in control subjects (n=270) (2.6%; P=0.033). In the HERITAGE cohort, the *His111 allele* was associated with a lower insulin sensitivity index (P=0.018) and a higher acute insulin response to glucose (P=0.0098) in Whites. Other variants showed associations with adiposity and plasma lipid values only in Blacks. Among Blacks, the *IVS2+G62T variant* was associated with body fat (P=0.002) and total cholesterol values (P=0.005), and the *Gly15Gly variant* with cholesterol (P=0.009) and triglyceride (P=0.05) levels. The haplotype derived from these two polymorphisms was associated with total body fat, while the *IVS2+G62T* and *Tyr111His*–haplotype was associated with body fat and disposition index.

Conclusions. The carriers of the *His111 allele* may have a higher risk of developing Type 2 diabetes mellitus. Racial differences were found between Blacks and Whites in body composition and lipids according to ACDC genotypes. Sequence variants in the adiponectin gene appear to be associated with diabetes and diabetes-related phenotypes.

Key words: *Adiponectin, gene, insulin sensitivity, linkage, type 2 diabetes*

Introduction

Adiponectin, a novel polypeptide encoded by apM1 mRNA (1), is the most abundant gene transcript (2) in adipose tissue. A decreased expression of adiponectin has been shown to correlate with insulin resistance in mouse models of altered insulin sensitivity (3). Importantly, recent data suggest that disruption of adiponectin causes insulin resistance (4,5) while adiponectin therapy exerts beneficial effects on glucose and insulin metabolism (3,6–7). Obese monkeys with lower plasma adiponectin levels exhibited reduced insulin-stimulated peripheral glucose uptake (8). Plasma adiponectin levels have been shown to be low in a prediabetic state suggesting that hypoadiponectinemia may be related to the development of insulin resistance (8).

In humans, inverse relationships between fasting insulinemia, insulin resistance, and plasma adiponectin levels have been observed (9,10). The expression of adiponectin is under feedback

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Abbreviations

$AIR_{Glucose}$	acute insulin response to glucose
BMI	body mass index
DI	disposition index
IVGTT	intravenous frequent sampling glucose tolerance test
Kg	glucose disappearance index
SI	insulin sensitivity index

inhibition in human obesity, and body weight loss increases plasma levels of adiponectin (11). The presence of Type 2 diabetes seems to decrease adiponectin levels even more (9). Adiponectin mRNA levels have been shown to be reduced in omental and subcutaneous adipose tissue of obese patients with Type 2 diabetes mellitus compared with lean and obese normoglycemic patients (12). High concentrations of adiponectin are independently associated with a reduced risk of Type 2 diabetes in healthy individuals (13) and predict increased insulin sensitivity (14).

The human adiponectin gene (ACDC=adipocyte, C1Q and collagen domain containing) is located on chromosome 3q27 (1), and recently two independent groups have reported significant evidence of linkage for obesity and diabetes-related traits with the region of chromosome 3 that contains the adiponectin structural gene (15,16). In the present study, we investigated whether polymorphisms in the adiponectin gene are associated with insulin metabolism and body fat distribution in the HERITAGE Family Study cohort. In addition, we compared the allele frequencies at ACDC sequence variants between diabetic and control subjects from a Finnish population.

Key messages

- The *His111 allele* of the adiponectin gene might be associated with a higher risk of type 2 diabetes in Whites.
- *Gly15Gly* and *IVS2+G62T* variants were associated with BMI and lipids among Blacks.
- The haplotype derived from these two polymorphisms was associated with body fat while the haplotype *IVS2+G62T – Tyr111His* was associated with body fat and disposition index.

Subjects, materials and methods

Subjects

The HERITAGE Family Study is a multicenter clinical trial conducted at five institutions. The specific aims, design, inclusion and exclusion criteria and methodology of the study have been described in detail elsewhere (17). The sample for the present study consists of 779 subjects, including 503 Whites and 276 Blacks. To be enrolled in the study, the individuals were required to be in good health (i.e. free of diabetes, cardiovascular diseases, or other chronic diseases) and to be sedentary at baseline (defined as no regular strenuous physical activity over the previous six months). The study protocol had been previously approved by each of the Institutional Review Boards of the HERITAGE Family Study research consortium. Written informed consent was obtained from each participant.

We also used 257 (134 women and 123 men) Finnish Type 2 diabetic individuals to compare the prevalences of various adiponectin gene polymorphisms against 285 non-diabetic, healthy Finnish control subjects. The diabetic subjects had been referred by general practitioners to the diabetic clinic of the Oulu University Hospital for an evaluation of diabetes treatment (18). Their mean age was 58.2 years. The control sample consisted of non-diabetic healthy women (n=140) and men (n=145). Their mean age was 55.7 years and they were selected from a random population cohort of 40-60 year-old individuals (19).

Phenotype measurements

In the present study, analyses were performed only with baseline phenotype data of the HERITAGE cohort. Body composition measures included hydrostatic weighing determination of body density, from which fat mass and fat-free mass were estimated (20). The sum of eight skinfolds (SF8) was used to assess the level of subcutaneous fat. Abdominal fat was quantified by computerized tomography as described by Sjöström et al. (21).

The intravenous frequent sampling glucose tolerance test (IVGTT) protocol was performed in the morning after an overnight (12 h) fast as described earlier (22). The glucose disappearance index (Kg) used as a measure of overall glucose tolerance, is defined as an estimate of the disappearance rate (%/min) of plasma glucose based on the slope of the line derived from least-squares regression of the natural logarithm of plasma glucose on time, from 10 through 60 min during the IVGTT. The acute insulin response to glucose ($AIR_{Glucose}$) was computed as the incremental integrated area under the

insulin curve for the first 10 min of the IVGTT and was used as an index of insulin secretion. The insulin sensitivity index (SI) represents the increase in net fractional glucose clearance rate per unit change in plasma insulin concentration after the intravenous glucose load. The disposition index (DI), derived as the product of SI and $AIR_{Glucose}$, is a measure of the activity of the beta cells adjusted for insulin resistance (23). All these variables were derived from the Minimal Model analysis (24). Because of skewed distributions, $AIR_{Glucose}$, SI and DI were transformed using a natural logarithm.

Plasma samples were collected under fasting conditions. Glucose and insulin analyses were performed as described earlier (22). Human serum adiponectin was measured with a commercial radioimmunoassay (Linco Research, Inc., Missouri, USA).

Genotyping

The polymerase chain reaction of the region containing the *IVS2+G62T polymorphism* in intron 2 and the *T→G transition (Gly15Gly)* at nucleotide 45 within exon 2 was performed using primers 5′-GAGTCCTTTGTAGGTCCCAAC-3′ and 5′-CTTTCTCCCTGTGTCTAGGC-3′. We used Dynazyme II (Finnzymes, Espoo, Finland) as a DNA-polymerase, 62°C as an annealing temperature and 1.5 mM $MgCl_2$-concentration. The *G276T polymorphism* was detected with the restriction enzyme *BsaMI* (Promega, Helsinki, Finland) and the *T45G polymorphism* with *Sma*I (Amersham, Espoo, Finland). The polymerase chain reaction for the *Tyr111His polymorphism* was done with primers 5′-CTGTTCTTTGTAGTCACTGAGG TC-3′ and 5′-GGAGGCCTTTAGATATTAT TC-3′. The DNA-polymerase was Ampli Taq (Applied Biosystems, Espoo, Finland), annealing temperature 57°C and $MgCl_2$ concentration 1.5 mM. The *T→C–polymorphism* in the sequence was detected with the enzyme BstZ17I (New England Biolabs, Espoo, Finland).

Statistical analysis

All analyses were performed with the SAS statistical software package (SAS Institute Inc., Cary, NC). A chi-square test was performed to determine whether the genotype frequencies were in Hardy-Weinberg equilibrium and to compare the allele and genotype frequencies between the diabetics and controls. All the insulin indices were adjusted for age, sex and fat mass. Because of the significant correlations between fat mass and abdominal fat phenotypes, the latter were adjusted for total fat mass, age and sex. Association analyses were carried out using a MIXED procedure in SAS. All the family members were included in the analyses. Non-independence among family members was adjusted for using a 'sandwich estimator', which asymptotically yields the same parameter estimates as ordinary least squares or regression methods, but the standard errors and consequently hypothesis tests are adjusted for the dependencies. The method is general, assuming the same degree of dependency among all members within a family. The analyses were performed separately for Blacks and Whites.

Linkage disequilibrium between pairs of SNPs was tested using the GENEPOP v.3.3 (2001) program (http://wbiomed.curtin.edu.au/genepop/) (25) for cross-tabulation and the EH version 1.14 for calculating linkage disequilibrium (http://linkage.rockefeller.edu/software/eh) (26).

Only parents were used for these analyses in the HERITAGE cohort, and values were calculated separately in Blacks and Whites. Haplotype frequencies and their differences between the Finnish type 2 diabetic and the control samples were calculated with a program available on the internet (version 2.1) at http://www.stat.washington.edu/stephens/phase/download.html(27,28). Haplotypes in the HERITAGE cohort were obtained using family structures and parental genotypes. Rare haplotypes ($n<9$) were not included in these statistical analyses.

Results

There was evidence of linkage disequilibrium among the three SNPs ($D'>0.9$), except for SNPs *IVS2+G62T* and *Gly15Gly* in the Finnish cohort ($D'=0.198$, $r^2=0.0009$). However, the low r^2 values, which better reflect the level of similarity between two SNPs especially in small populations, ($r^2<0.04$ among all polymorphisms) suggest that the SNPs are largely in linkage equilibrium.

The genotype distributions and allele frequencies of the three polymorphisms based only on parents are presented in Table I. No differences in genotype or allele frequencies were observed either between Whites and Blacks or between males and females. All genotype frequencies were in Hardy-Weinberg equilibrium.

Body composition phenotypes in relation to the ACDC genotypes are shown in Table II. In Blacks but not in Whites, the *IVS2+G62T polymorphism* was associated with percentage body fat ($P=0.002$) and subcutaneous fat as assessed by sum of eight skinfolds ($P=0.014$). The *G62G* subjects had the highest amount of total and subcutaneous fat.

Table I. Genotype and allele frequencies for the ACDC polymorphisms in the HERITAGE cohort.

Polymorphism	Genotype frequency			Allele frequency	
Tyr111His	Tyr111Tyr	Tyr111His	His111His	Tyr	His
Blacks	0.99 (76)	0.01 (1)	0.00 (0)	0.99	0.01
Whites	0.91 (174)	0.08 (15)	0.01 (1)	0.95	0.04
IVS2+G62T	G62G	G62T	T62T	G	T
Blacks	0.48 (37)	0.44 (34)	0.08 (6)	0.70	0.30
Whites	0.54 (102)	0.41 (78)	0.05 (10)	0.74	0.26
Gly15Gly	T45T	T45G	G45G	T	G
Blacks	0.87 (67)	0.12 (9)	0.01 (1)	0.93	0.07
Whites	0.81 (153)	0.18 (35)	0.01 (2)	0.90	0.10

Number of subjects in parentheses. Computations based only on parents.

G62G Black subjects at the *IVS2+G62T polymorphism* showed higher total cholesterol ($P=0.005$ for trend) than *G62T* subjects (Table III). The *Gly15Gly polymorphism* was also associated with lipid values in Blacks. The *G allele* carriers registered higher total cholesterol ($P=0.009$) and total triglycerides ($P=0.05$) than *G allele* non-carriers. None of the polymorphisms were associated with computerized tomography-measured abdominal fat phenotypes (data not shown).

Glucose metabolism-related phenotypes by *Tyr111His genotypes* in Whites are seen in Table IV and Figure 1. Fasting plasma glucose did not differ between genotypes, and although plasma fasting insulin tended to be higher in *His* carriers, it was not significant (Table IV). The number of subjects ($n=37$) who had plasma glucose in the range of 6.1 to 6.9 mmol/l (impaired fasting blood glucose) was not different between genotype groups (data not shown). However, SI was lower ($P=0.018$) in *His111* carriers at the *Tyr111His polymorphism* than in *His allele* non-carriers (Figure 1). Furthermore, $AIR_{glucose}$ was higher in carriers ($P=0.0098$) than in non-carriers of the *His111 allele*. Similar findings were observed for the insulin variables when only independent individuals were considered (data not shown). The DI did not show any significant differences between genotype groups. In Blacks, the frequency of *Tyr111His* subjects ($n=4$) was too low to make comparisons although trends similar to

Table II. Body composition phenotypes in relation to the ACDC genotypes in the HERITAGE cohort.

Polymorphism/genotype	Body mass index	*P*	Percentage body fat	*P*	Subcutaneous fat	*P*
Tyr111His						
Blacks						
Tyr111His/His111His ($n=4$)	26.9 (1.9)	NS*	18.9 (2.9)	NS	121.0 (32.6)	NS
Tyr111Tyr ($n=212–272$)	28.0 (0.9)		24.9 (1.7)		141.9 (7.2)	
Whites						
Tyr111His/His111His ($n=38–39$)	25.6 (0.8)	NS	22.0 (1.7)	NS	158.0 (11.5)	NS
Tyr111Tyr ($n=427–464$)	25.9 (0.4)		21.3 (1.0)		150.8 (6.0)	
IVS2+G62T						
Blacks						
T62T ($n=19–23$)	26.8 (1.3)	NS	19.9 (2.4)	0.002*	123.5 (12.4)	0.014†
G62T ($n=89–117$)	27.5 (0.9)		22.8 (1.8)		132.0 (7.9)	
G62G ($n=106–136$)	28.8 (1.0)		27.4 (1.9)		153.6 (8.0)	
Whites						
T62T ($n=24–25$)	26.0 (0.9)	NS	20.5 (2.1)	NS	150.6 (11.9)	
G62T ($n=187–203$)	25.8 (0.4)		21.2 (1.1)		149.6 (6.0)	NS
G62G ($n=254–275$)	26.1 (0.4)		21.5 (1.1)		153.0 (7.1)	
Gly15Gly						
Blacks						
T45T ($n=195–247$)	28.0 (0.9)	NS	24.9 (1.8)	NS	141.2 (7.4)	NS
T45G+G45G ($n=1–29$)	28.3 (1.1)		25.1 (2.6)		150.9 (14.4)	
Whites						
T45T ($n=373–401$)	26.0 (0.4)	NS	21.6 (1.1)	NS	152.8 (6.4)	NS
T45G+G45G ($n=92–102$)	25.7 (0.5)		20.2 (1.2)		144.5 (6.8)	

*NS=not significant. Values are means (SE). Adjusted for age and sex. All the family members were included in the analyses. * $P=0.001$ between G62G and T62T; $P=0.0076$ between G62G and G62T. † $P=0.0126$ between G62G and T62T; $P=0.0182$ between G62G and G62T.

Table III. Plasma lipids in relation to ACDC genotypes in the HERITAGE cohort.

Polymorphism/genotype	Total cholest-erol (mmol/l)	*P*	HDL-cholesterol* (mmol/l)	*P*	Total TG* (mmol/l)	*P*
Tyr111His						
Blacks						
Tyr111His/His111His (*n*=4)	3.83 (0.16)	NS	1.10	NS	0.65	NS
Tyr111Tyr (*n*=268)	4.30 (0.10)		1.06		0.92	
Whites						
Tyr111His/His111His (*n*=39)	4.38 (0.16)	NS	0.94 (0.08)	NS	1.14 (0.22)	NS
Tyr111Tyr (*n*=463)	4.38 (0.09)		0.97 (0.04)		1.18 (0.12)	
IVS2+G62T						
Blacks						
T62T (*n*=23)	4.25 (0.18)	0.005*	1.03 (0.11)	NS	0.90 (0.20)	NS
G62T (*n*=116)	4.15 (0.09)		1.05 (0.07)		0.90 (0.11)	
G62G (*n*=133)	4.50 (0.11)		1.08 (0.08)		0.95 (0.14)	
Whites						
T62T (*n*=25)	4.29 (0.20)	NS	0.99 (0.06)	NS	1.23 (0.26)	NS
G62T (*n*=203)	4.38 (0.10)		0.97(0.04)		1.16 (0.12)	
G62G (*n*=274)	4.38 (0.10)		0.96 (0.05)		1.20 (0.12)	
Gly15Gly						
Blacks						
T45T (*n*=245)	4.26 (0.10)	0.009	1.06 (0.07)	NS	0.91 (0.13)	0.05
T45G+G45G (*n*=27)	4.78 (0.16)		1.07 (0.10)		1.06 (0.20)	
Whites						
T45T (*n*=400)	4.38 (0.09)	NS	0.96 (0.04)	NS	1.19 (0.12)	NS
T45G+G45G (*n*=102)	4.35 (0.11)		0.98 (0.06)		1.13 (0.13)	

Values are means (SE). Adjusted for age and sex. All the family members were included in the analyses. *P=0.001 between G62G and G62T. * HDL=high density lipoprotein; TG=triglycerides.

those in Whites in insulin-related phenotypes were seen (data not shown). Other polymorphisms were not associated with glucose or insulin phenotypes (data not shown).

Serum adiponectin levels were analyzed in White carriers of the *Tyr111His* [*His111His* (*n*=1) plus *Tyr111His* (*n*=38)] mutations, and were compared to the levels in age-, sex- and body fat-matched White subjects without the mutation (*n*=39). The mean serum adiponectin levels tended to be lowest in a *His111His* subject (*n*=1) [1.81 μg/ml], intermediate in *Tyr111His* subjects (*n*=38) [4.23 μg/ml (0.75) (SE)] and the highest in *Tyr111Tyr* subjects (*n*=39) [6.24 μg/ml (0.78)] (*P*=0.2 when *His111His* subjects were combined with *Tyr111His* subjects).

Genotype and allele frequencies for the ACDC polymorphisms between Finnish Type 2 diabetic subjects and controls are shown in Table V. The *His111 allele* frequency was higher (5.1%) in diabetic than in control subjects (2.6%; *P*=0.033). Other polymorphisms did not differ between diabetics and controls.

We estimated the frequencies of the *Gly15Gly*, *IVS2+G62T* and *Tyr111His* haplotypes in Finnish type 2 diabetic and control subjects and found no differences in haplotype frequencies between the two groups. These results are shown in Table VI.

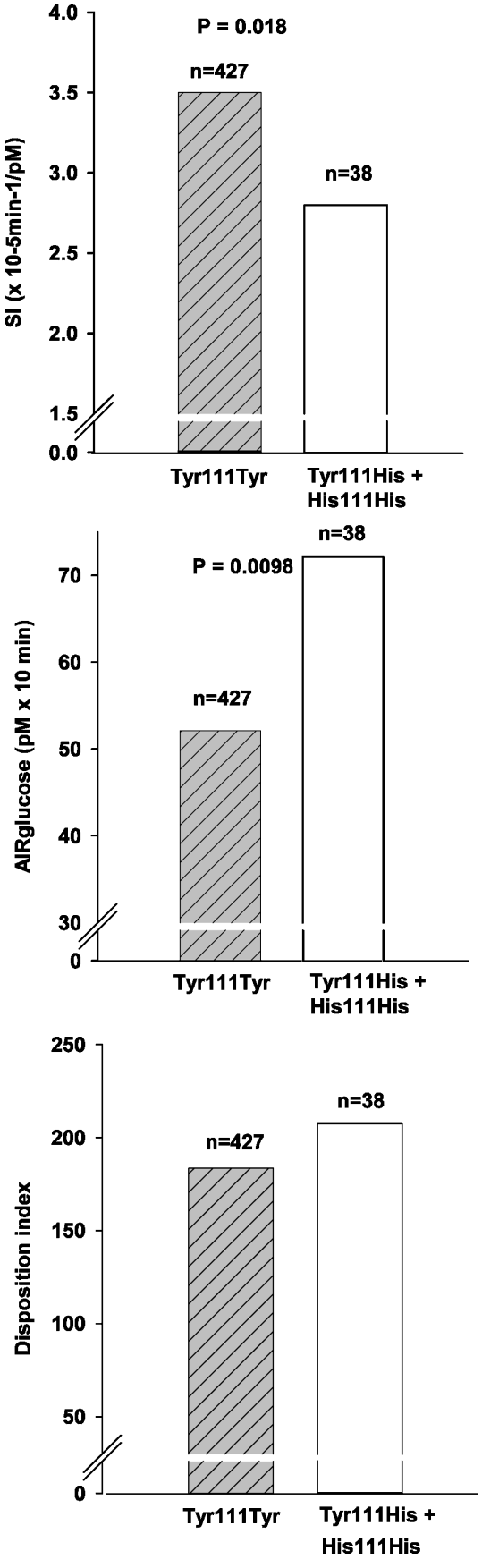
Haplotypes defined by the *Gly15Gly* and *IVS2+T62G* polymorphisms were associated with body fat in the Black subjects of HERITAGE. Blacks with the tg/tg genotype (*n*=98) had a mean body fat of 28.7% whereas the other genotypes had mean body fat values of 23.8% (tg/gg *n*=9) and 21.3% (tt/tt *n*=19) (*P*=0.009).

The haplotypes derived from the *IVS2+G62T* and *Tyr111His* polymorphisms were associated with

Table IV. Glucose metabolism phenotypes by ACDC *Tyr111His polymorphism* in the HERITAGE cohort.

Genotype	Fasting insulin	Fasting glucose	K_g
Tyr111His+His111His (*n*=38)	64.3 (14.2)	95.6 (2.7)	1.61 (0.11)
Tyr111Tyr (*n*=427)	52.7 (7.1)	94.3 (1.6)	1.53 (0.05)
P-value	NS	NS	NS

Values are logarithmic backtransformed means (SE). Fasting plasma glucose is adjusted for age, sex. Fasting insulin and K_g are also adjusted for fat mass. All the family members were included in the analyses.



body fat and disposition index in Blacks. The genotypes gT/gT had the highest body fat (27.9%, n=117) and lowest disposition index (mean 2.40). The mean body fat of gT/gH heterozygotes was 21.8% (n=43) and disposition index 2.57. The tT/tT genotype had the lowest body fat (18.5%, n=19) and highest disposition index (2.66). Differences between haplotypes were statistically significant (P=0.046).

In the HERITAGE study, there were no statistically significant associations between haplotypes constructed from the *Tyr111His, Gly15Gly* and *IVS2+G62T* polymorphisms and insulin indexes or body fat.

Discussion

In a Japanese population, Takahashi et al. (29) reported one case with a missense mutation in codon 112 of exon 3 in the ACDC gene. An adjacent mutation causing a change of a tyrosine to histidine in codon 111 was not shown to be associated with obesity in our earlier report (30). However, the latter is located in the globular domain of the adiponectin protein and was associated with indices of carbohydrate metabolism in the current study. In the HERITAGE cohort, *His111 allel*e carriers showed lower SI indicating that they were more insulin resistant than non-carriers. In addition, presumably as a compensatory mechanism, *His111* carriers had higher acute insulin response to glucose. As the increase in AIR was appropriate for the degree of insulin resistance, the DI was not significantly different among the genotype groups. Thus the presence of the *His111 allele* was not associated with apparent beta cell dysfunction. Because the *His111 allele* carriers are more insulin resistant, it is possible that beta cell compensation may ultimately collapse in some individuals, reflecting an increased risk of developing Type 2 diabetes mellitus. The latter notion was confirmed in the Oulu diabetic study where the *His111 allele* frequency was higher in Type 2 diabetic subjects than in controls.

In earlier studies, the functional effects of the Tyr111His mutation were investigated. Waki et al. (2003) reported that while the Tyr-His substitution showed no changes in multimer formation as compared with the wild-type, the R112C mutant showed severely impaired trimer formation (31). The mean serum adiponectin levels tended to be lower in White *His111 allele* carriers, who were also

Figure 1. Glucose metabolism phenotypes in relation to *Tyr111His genotypes* in Whites in the HERITAGE cohort. All the family members were included in the analyses.

Table V. Genotype and allele frequencies for the ACDC polymorphisms in Finnish Type 2 diabetics and controls.

	Genotype frequency			Allele frequency	
Tyr111His *	Tyr111Tyr	Tyr111His	His111His	Tyr	His
Diabetics (n=254)	229 (0.902)	24 (0.094)	1 (0.004)	0.949	0.051
Controls (n=270)	256 (0.948)	14 (0.052)	0 (0.0)	0.974	0.026
Gly15Gly (T45G) #	T45T	T45G	G45G	T	G
Diabetics (n=258)	235 (0.911)	23 (0.089)	0 (0.0)	0.955	0.045
Controls (n=283)	255 (0.901)	26 (0.091)	2 (0.007)	0.947	0.053
IVS2+G62T #	G62G	G62T	T62T	G	T
Diabetics (n=255)	116 (0.455)	104 (0.408)	35 (0.137)	0.659	0.341
Controls (n=283)	124 (0.438)	124 (0.438)	35 (0.124)	0.657	0.343

*P=0.034 for the genotype frequencies between diabetics and controls. P=0.033 for the alleles frequencies between diabetics and controls.
No statistically significant results

more insulin resistant than *Tyr111Tyr* subjects, supporting an earlier study (32). In both animals (8) and humans (14), low plasma adiponectin levels have been correlated with insulin resistance (8) while high concentrations of adiponectin are independently associated with a reduced risk of Type 2 diabetes in healthy individuals. These findings are consistent with the concept that prolonged insulin resistance provides a 'stress' to pancreatic beta cells, which may fail over time, leading to Type 2 diabetes.

In a recent study of Swedish Caucasians, there was no association between the Tyr111His polymorphism and type 2 diabetes or impaired glucose tolerance (33). However, the frequency of the His allele is very low, which may have reduced the ability to obtain statistically significant effects. In addition, the impact of the genotype on the phenotypes of interest appears to be small and may be dependent on the genetic background of the population.

In the Japanese population, the *IVS2+G62T* variant of the ACDC gene has been associated with type 2 diabetes and insulin resistance (34). In the study by Filippi (35), IVS2+G62T (276G>T) was associated with higher BMI, plasma insulin and insulin resistance. In the study of Swedish Caucasians, there was no association between *IVS2+G62T* and type 2 diabetes or insulin resistance (33). The *IVS2+G62T polymorphism* was not associated with insulin indices in the present study. However, *G62G* subjects had a higher amount of total and subcutaneous fat than other genotypes. Inverse relationships between adiponectin serum levels and overall adiposity (36) or visceral fat (37) have been observed earlier. It has been suggested that the contribution of visceral fat accumulation to insulin resistance may be mediated at least partly through hypoadiponectinemia (37). The associations in the present study were observed only among

Table VI. Haplotype frequencies derived from ACDC polymorphisms in Finnish Type 2 diabetics and controls and Blacks and Whites of the HERITAGE cohort.

Haplotype	Finnish control group	Finnish diabetics	HERITAGE Whites	HERITAGE Blacks
Gly15Gly − IVS2+T62G				
tg	0.61	0.62	0.65	0.72
tt	0.34	0.33	0.25	0.25
gg	0.05	0.04	0.10	0.03
Gly15Gly− Tyr111His				
tH	0.02	0.05	0.08	0.00
tT	0.93	0.90	0.87	0.97
gT	0.05	0.05	0.09	0.02
IVS2+G62T − Tyr111His				
gH	0.02	0.05	0.04	0.01
gT	0.63	0.61	0.71	0.76
tT	0.35	0.34	0.25	0.24
Gly15Gly − IVS2+G62T− Tyr111His				
ttT	0.34	0.33	0.25	0.25
tgH	0.02	0.05	0.04	0.01
tgT	0.58	0.57	0.61	0.72
ggT	0.05	0.04	0.10	0.05

Blacks in whom the *G62G genotype* was also associated with higher total cholesterol. The effect of this variant on plasma lipid values seems to be mediated through its effects on total adiposity since the association was no longer significant when adjusted for fat mass. An earlier study suggested adiponectin to be a link between body fat distribution, insulin resistance and an atherogenic lipoprotein profile (38). The *IVS2+G62T polymorphism* is not located in a coding region of the ACDC gene, and the functional implications of the variant need to be further investigated.

A third variant, *Gly15Gly*, within exon 2 of the ACDC gene has been associated with obesity and insulin sensitivity in a German population, with *G allele* carriers having the highest value. However, the effect of this variant on obesity risk was observed only in individuals without a familial predisposition to Type 2 diabetes (39). This SNP did not associate with BMI or insulin resistance in another study (35) or with insulin resistance in Swedish Caucasians (33). In the present study, the *Gly15Gly polymorphism* was not associated with obesity but was associated with cholesterol and triglyceride levels in Blacks, with *G allele* carriers showing the highest values. This is in accordance with an earlier study in a German population (40). The mechanisms hypothesized to influence fat and glucose metabolism may also affect plasma lipid concentrations. Adiponectin has been shown to increase lipid oxidation in muscle and other tissues (3,41). The *Gly15Gly polymorphism*, located in the non-helical region of ACDC, does not change the amino acid composition of adiponectin protein but its effects could be mediated by linkage disequilibrium with another functional mutation elsewhere in the same gene or in another gene.

The r^2 values were small ($r^2 < 0.04$) among all SNPs of this study. Thus the alleles are in linkage equilibrium. Haplotype frequencies did not differ between the Finnish type 2 diabetics and controls. An association between *IVS2+G62T - Gly15Gly* haplotype tg and low adiponectin levels, and a higher risk of diabetes was reported earlier (42). We could not find any evidence in support of these observations in our study. A lack of association between the tg haplotype and diabetes was also reported in a recent report (43). However, in our study, the ACDC haplotypes associated with certain obesity-related phenotypes in Blacks of the HERITAGE cohort. A haplotype (tg-) derived from the *IVS2+G62T* and *Gly15Gly* polymorphisms was associated with obesity in Blacks. The *Gly15Gly–IVS2+G62T* haplotype tg- was also associated with obesity and other features of the insulin resistance syndrome in two other studies (35,43). In the present study, the *IVS2+G62T* and *Tyr111His* haplotype gT was associated with a higher body fat and lower disposition index. These associations may reflect the independent effects of *IVS2+G62T* and *Gly15Gly* variants, or may be the consequence of other nearby polymorphisms.

It is commonly recognized that African-Americans have higher rates of obesity and associated morbidities than Caucasians. One could speculate that sequence variation in the adiponectin gene might be involved in these racial differences in metabolism. In fact, we observed differences between Blacks and Whites in body composition and lipids according to the *IVS2+G62T* and *Gly15Gly* genotypes and haplotypes. The interactions of genotypes and environmental factors on these phenotypes may differ between the two ethnic groups. Differences in the effects of SNPs in the ACDC gene among populations and ethnic groups could result from variation in genetic backgrounds and haplotype structures (33).

In conclusion, the *Tyr111His polymorphism* was associated with insulin sensitivity and acute insulin response to glucose in Whites. The carriers of the *His111 allele* were at higher risk of developing Type 2 diabetes and tended to have a lower serum adiponectin level in Whites. The *IVS2+G62T variant* was associated with adiposity and cholesterol values among Blacks, while the *Gly15Gly variant* was associated with cholesterol and triglyceride levels also in Blacks. The haplotype tg derived from the *Gly15Gly* and *IVS2+G62T polymorphisms* was associated with body fat, while haplotypes derived from the *IVS2+G62T* and *Tyr111His polymorphisms* were associated with body fat and disposition index among Blacks. These results provide more evidence to the effect that DNA sequence variations in the adiponectin gene are associated with obesity-related phenotypes.

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