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The W64R variant of the β_3-adrenergic receptor is not associated with Type II diabetes or obesity in a large Finnish sample

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Summary Recent studies have suggested an association between Type II (non-insulin-dependent) diabetes mellitus-related phenotypes and a cytosine-to-thymidine substitution that results in the replacement of tryptophan by arginine at codon 64 (Trp64Arg or W64R) of the β_3-adrenergic receptor gene. Here, we present the results of possibly the largest association study to date on the variant in a sample of 526 families with a total of 1725 subjects, 1053 of whom had Type II diabetes. Preliminary calculations suggested that we had excellent power to detect the moderate associations which were reported in previous studies. No associations were found between the W64R variant and the following phenotypes in our sample: Type II diabetes, age at diagnosis for Type II diabetes, measures of obesity, fasting glucose, fasting insulin, minimal model variables, and systolic and diastolic blood pressures. In the analysis of plasma lipids, we detected an association between the variant and HDL ratios (HDL cholesterol/total cholesterol) ($p = 0.013$), which remained significant even after adjusting for sex, affection status and age. Since W64R homozygotes ($n = 11$) had the highest HDL ratios, however, heterozygotes had the lowest and the wild-type subjects had intermediate values, we conclude that the W64R variant is unlikely to reduce HDL ratios in a dose-dependent, pathogenic manner. [Diabetologia (1999) 42: 238–244]

Keywords β_3-Adrenergic receptor, Type II diabetes, obesity, association.

Obesity affects up to one third of people in Western populations and severe obesity is associated with excess morbidity and mortality [1]. Obesity increases insulin resistance which often predisposes to Type II (non-insulin-dependent) diabetes mellitus even though the mechanisms for this pathway are still not clear [2–5].

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Abbreviations: S_I, Insulin sensitivity; S_G, glucose effectiveness; DI, disposition index; $AIR_{glucose}$, acute insulin response to glucose; W64R or Trp64Arg, replacement of Tryptophan (W) by Arginine (R) at codon 64 of the β_3-Adrenergic receptor gene; GEE, generalized estimating equations; OR, odds ratio; CI, confidence interval; FUSION, The Finland-US Investigation of NIDDM; PCR, polymerase chain reaction.

There is evidence that functional β_3-adrenergic receptors are present in both human white and brown adipose tissue [6, 7] and β_3-adrenergic receptor agonists have been shown to decrease insulin resistance in both obese diabetic and non-diabetic subjects [8]. Targeted disruption of the β_3-adrenoceptor gene has given rise to animals that are generally more obese [9, 10]. Thus, it is not surprising that a number of studies have focused on the role of β_3-adrenergic receptor variants in Type II diabetes and obesity. Altered function could promote obesity by increased fat storage in white adipose tissue and decreased thermogenesis in brown adipose tissue. Further, abnormal β_3-adrenergic receptor activity could result in increased insulin resistance by increasing delivery of non-esterified fatty acids from intra-abdominal fat stores to the portal circulation [11].

Recently, a β_3-adrenergic receptor variant was found to be associated with insulin resistance [12],

rate of weight gain [13], and earlier age-of-onset for Type II diabetes [14, 12]. The variant replaces a thymidine (TGG) with a cytosine (CGG) at nucleotide position 190 causing a change from tryptophan to arginine at codon 64. The evidence for most of these associations was modest. These studies prompted us to investigate the role of this β_3-adrenergic receptor missense variant in Type II diabetes, Type II diabetes-related traits and obesity in a large Finnish cohort. We believe this represents the largest single analysis of the variant to date.

Materials and methods

FUSION study design. The Finland-US Investigation of NIDDM (Type II diabetes mellitus) Genetics (FUSION) Study is an international collaborative effort with the goal of mapping and cloning the genes predisposing to Type II diabetes and intermediate quantitative traits in Finnish subjects [15]. The age at diagnosis of Type II diabetes in index cases from which the families were ascertained ranged from 35–60 years. In addition to Type II diabetes disease status, several Type II diabetes-associated metabolic traits have been measured in unaffected spouses and offspring of index subjects (or alternatively, of affected siblings) by frequently sampled intravenous glucose tolerance tests (FSIGT) and analysis using a minimal model approach [16]. Anthropometric measurements of all subjects were determined by standard methods.

We analysed 1035 affected siblings, 24 mothers (of whom 15 were affected), 7 fathers (3 affected), 180 unaffected spouses, 479 unaffected offspring of affected subjects and 219 unrelated, unaffected control subjects. Prior to analysis we excluded families in which either a sibling or first-degree relative had Type I (insulin-dependent) diabetes mellitus, affected spouses or affected offspring and those people whose diabetic status was not known. The following traits were analysed: age at diagnosis of Type II diabetes, body mass index (BMI), waist-to-hip ratio, weight at 20 years of age, current weight, change in weight relative to weight at 20 years of age, maximum weight, maximum lifetime weight change relative to weight at 20 years of age, fasting glucose, fasting insulin, insulin sensitivity (S_I), glucose effectiveness (S_G), acute insulin response ($AIR_{glucose}$), the disposition index (DI, which is [$AIR_{glucose} \times S_I$]), triglycerides, total cholesterol, HDL, HDL ratio (HDL cholesterol/total cholesterol), systolic blood pressure (mean of two measurements) and diastolic blood pressure (mean of two measurements). We also formulated two standardized weight measures which give an estimate for rate of weight gain, a trait found to be associated with the variant in one of the original reports [13]; the standardized weight change relative to weight at 20 years of age

$$\frac{cwt - wt\,20}{wt\,20 \times (age - 20)},$$

where cwt = current weight and wt20 = weight at 20 years of age and the standardized maximum weight change relative to weight at 20 years,

$$\frac{\max wt - wt\,20}{wt\,20 \times (age - 20)},$$

where maxwt = maximum recorded weight. These formulae represent the proportional changes in weight per year past age 20 years. Diabetic people were analysed for every trait except for the minimal model measures. Additional details of the FUSION study design including the criteria for selecting out families with Type I diabetes can be found in an earlier report [15]. Family studies were approved by the institutional review boards at the National Institute of Health, USA (assurance number SPA S-5737–05) and at the National Public Health Institute in Helsinki, Finland.

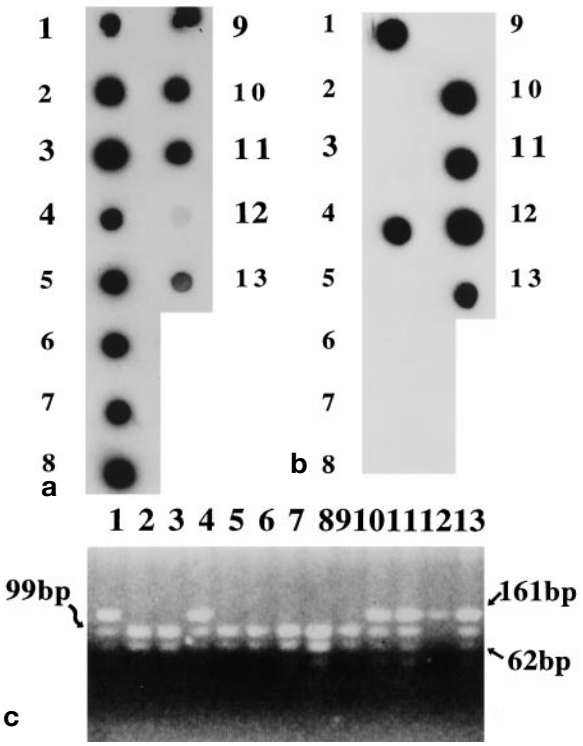

Fig. 1 a, b, c. W64R variant detection by hybridization with allele-specific oligonucleotides (ASO) and restriction enzyme digestion using *BstN*1. **a** shows a dot-blot with PCR samples 1–13 hybridized with a probe for the wild-type allele. Samples 1–11 and 13 are positive and sample 12 is negative for the presence of this allele. **b** shows the same blot which was stripped and then hybridized with the W64R probe. Samples 1, 4, 10–13 are positive and the other PCR samples are negative for the presence of the variant allele allowing genotypes to be assigned when used in conjunction with results from **a**. These are wild-type genotypes (2, 3, 5–9), heterozygote genotypes (1, 4, 10, 11 and 13) and W64R genotypes (12). **c** shows a restriction digest of a 210 bp PCR product containing the variant polymorphism. The presence of the variant allele abolishes the restriction site. Thus W64R homozygote subjects have two 161 bp fragments (lane 12, for sample 12), wild-type subjects have one 99 bp and one 62 bp fragment (lanes 2, 3, 5–9) whereas the heterozygote subjects yield three fragments of sizes 62 bp, 99 bp and 161 bp (lanes 1, 4, 10–13). All genotypes give additional bands of lengths 30, 12 and 7 bps which migrate out of the gel. These results concur with those of **a** and **b**

W64R variant detection. The presence or absence of the W64R variant was detected by two methods, allele-specific oligonucleotide hybridization and restriction digests after amplification of genomic DNA. Allele-specific oligonucleotide hybridizations were carried out on the total data set of 2401 samples whereas restriction digests were done on a random subset of 27% (645/2401) of the total.

Standard amplification of a 210 bp fragment of the β_3-adrenergic receptor gene was carried out in a total volume of

20 μl using the primers BSTNUP and BSTNDOWN [12] except that the polymerase chain reaction (PCR) mix contained 20 % sucrose and 0.1 mmol/l cresol red. The protocol reported previously [17] was followed for dot-blots using wild or variant radiolabelled probes [14]. Five microlitres of the amplified DNA was digested with *BstN*1 in a final volume of 15 μl at 60 °C for 2 h before being run on a standard agarose gel (Fig. 1).

Statistical analysis. To investigate the potential effect of the W64R polymorphism on each response while allowing for familial correlations, we used a generalized estimating equations approach [18]. Phenotype measures were transformed when necessary to approximate model assumptions. For each response we tested whether the mean response differed among the three genotypes (genotype analysis). We also tested whether the mean response differed between those people with and those without the W64R variant (allele analysis). Analyses were carried out both with and without covariates including age, sex, Type II diabetes status, BMI, smoking status and alcohol consumption. Selected interactions between the presence of the variant and the covariates were also included in the allelic analysis. All statistical tests computed test specific a priori hypotheses derived from positive associations reported in the literature [12–14, 19]. Hence we made no correction for multiple comparisons.

We estimated allele frequencies in both affected and unaffected family members taking into account the family structures [20, 21]. To compare the allele frequencies among the control subjects, spouses and siblings, we computed the standard z-test for proportions using the asymptotic standard error for siblings provided by MENDEL [20, 21].

We computed the approximate power of our sample to detect differences in trait values that have been reported previously [12–14, 19]; we also computed the differences detectable between those with and those without the W64R variant with approximately 90 % power for each of the traits discussed in this paper. Power estimates were determined by using the program PC-SIZE [22] which does not adjust for the within-family correlation.

We report results for people who, on the day of their examinations, did not take medications that could influence the trait of interest. Analyses were repeated excluding all those who were prescribed a drug that would be expected to influence the response variable (general exclusion analysis). We only report the latter results if they are significant.

Results

All genotypes ($n = 2401$) were scored by three independent observers. We excluded 204 genotypes (8.5 %) which could not be consistently scored by all three observers. For the remaining genotypes, five discrepancies were seen between the two methods for genotyping (5/645) giving a 99.2 % concordance rate (Fig. 1). Results of the dot-blot analysis were recorded and where possible, verified in the random sample of restriction digests. From the genotyped data set of 2197 people, 253 were then excluded (see Materials and methods).

Analysis of this final data set of 1725 family members yielded 1440 with the wild-type genotypes (WW), 273 heterozygotes (WR), and 12 W64R variant homozygotes (RR) (see Table 2). The correspond-

Table 1. β_3-adrenergic-receptor W64R variant: difference detectable with 90 % power in this study

Variable	Previously Reported Differences[a]	Detectable Difference	Sample Size
Age at diagnosis (years)	5[14, 12]	2.60	$n_{ww} = 867$ $n_{R-} = 162$
BMI (kg/m^2)	4[13]	1.02	$n_{ww} = 1428$ $n_{R-} = 280$
Waist-to-hip ratio	0.03[12]	0.02	$n_{ww} = 1421$ $n_{R-} = 280$
Weight at 20 years of age (kg)	–	2.35	$n_{ww} = 1373$ $n_{R-} = 272$
Current weight (kg)	14[13]	3.12	$n_{ww} = 1431$ $n_{R-} = 282$
Current weight minus weight at 20 years of age (kg)	16[13]	2.55	$n_{ww} = 1370$ $n_{R-} = 269$
Standardized weight change relative to weight at 20 years of age (years^{-1})	–	0.07	$n_{ww} = 1368$ $n_{R-} = 269$
Maximum weight (kg)	12[13]	3.59	$n_{ww} = 1424$ $n_{R-} = 285$
Maximum weight minus weight at 20 years of age (kg)	15[13]	2.94	$n_{ww} = 1373$ $n_{R-} = 272$
Standardized maximum weight change relative to weight at 20 years of age (years^{-1})	–	0.32	$n_{ww} = 1372$ $n_{R-} = 272$
Fasting plasma glucose (mmol/l)	–	0.65	$n_{ww} = 1393$ $n_{R-} = 275$
Fasting serum insulin (pmol/l)	–	10.45	$n_{ww} = 1076$ $n_{R-} = 205$
Glucose effectiveness ($10^{-2} \times$ min^{-1})	0.5[19]	0.002	$n_{ww} = 436$ $n_{R-} = 96$
Insulin sensitivity ($10^{-5} \times$ (min $\times$ pmol/l)$^{-1}$)	10.5[19]	1.23	$n_{ww} = 436$ $n_{R-} = 96$
Acute insulin response (pmol/l $\times$ 10 min)	–	1.06	$n_{ww} = 448$ $n_{R-} = 99$
Disposition index	–	2867.0	$n_{ww} = 432$ $n_{R-} = 96$
Triglycerides (mmol/l)	–	0.21	$n_{ww} = 1404$ $n_{R-} = 278$
Total cholesterol (mmol/l)	–	0.24	$n_{ww} = 1404$ $n_{R-} = 278$
HDL cholesterol (mmol/l)	–	0.07	$n_{ww} = 1404$ $n_{R-} = 278$
HDL ratio	–	0.0002	$n_{ww} = 1404$ $n_{R-} = 278$
Mean systolic blood pressure (mm Hg)	–	4.7	$n_{ww} = 1402$ $n_{R-} = 275$
Mean diastolic blood pressure (mm Hg)	4.0[12]	2.3	$n_{ww} = 1402$ $n_{R-} = 275$

n_{ww} and n_{R-} represent the number of subjects homozygous for the wild-type allele and either heterozygous or homozygous for the variant allele, respectively.

[a] Reported differences between subjects with and without the W64R variant (12, 13, 14, 19)

Table 2. Genotypic distribution of trait values for the W64R variant

Outcome	Genotypes	*n*	Means ± SD	Median	Allelic *p* value[a]	Genotypic *p* value[b]
Age at diagnosis (years)	WW	867	52.4 ± 9.2	52	0.85	0.52
	WR	155	52.4 ± 9.4	52		
	RR	7	54.9 ± 6.2	55		
BMI (kg/m^2)	WW	1428	28.5 ± 4.9	28.0	0.39	0.57
	WR	268	28.9 ± 5.0	28.7		
	RR	12	27.9 ± 5.6	27.4		
Waist-to-hip ratio	WW	1421	0.91 ± 0.09	0.91	0.80	0.76
	WR	268	0.91 ± 0.10	0.92		
	RR	12	0.89 ± 0.10	0.92		
Weight at 20 years of age (kg)	WW	1373	63.9 ± 11.5	63.0	0.53	0.56
	WR	262	64.5 ± 12.1	63.5		
	RR	10	66.6 ± 9.2	65.0		
Current weight (kg)	WW	1431	80.0 ± 15.4	79.0	0.65	0.83
	WR	270	81.0 ± 15.8	80.6		
	RR	12	77.7 ± 18.1	73.5		
Change in weight relative to weight at 20 years of age (kg)	WW	1370	16.4 ± 12.3	14.7	0.80	0.68
	WR	259	16.9 ± 12.1	16.7		
	RR	10	12.9 ± 17.3	7.4		
Standardized weight change relative to weight at 20 years of age (years^{-1})	WW	1368	0.0087 ± 0.0082	0.0071	0.55	0.81
	WR	259	0.0092 ± 0.0091	0.0081		
	RR	10	0.0099 ± 0.0153	0.0036		
Maximum weight (kg)	WW	1424	87.5 ± 18.0	86.0	0.72	0.93
	WR	273	87.8 ± 18.8	85.6		
	RR	12	85.2 ± 15.5	81.5		
Maximum weight change relative to weight at 20 years of age (kg)	WW	1373	23.8 ± 14.2	22.0	0.69	0.82
	WR	262	24.0 ± 13.7	23.0		
	RR	10	20.5 ± 14.9	18.5		
Standardized maximum weight change relative to weight at 20 years of age (years^{-1})	WW	1372	0.0125 ± 0.0098	0.0105	0.91	0.95
	WR	262	0.0129 ± 0.0010	0.0107		
	RR	10	0.0128 ± 0.0138	0.0080		
Fasting plasma glucose (mmol/l)	WW	1393	8.3 ± 3.6	7.3	0.63	0.78
	WR	263	8.2 ± 3.9	6.9		
	RR	12	8.7 ± 4.2	7.1		
Fasting serum insulin (pmol/l)	WW	1076	89.9 ± 56.4	78.0	0.16	0.33
	WR	195	87.7 ± 57.6	72.0		
	RR	10	75.6 ± 40.4	63.0		
Glucose effectiveness ($10^{-2} \times$ min^{-1})	WW	436	1.74 ± 0.57	1.68	0.94	[c]
	WR	95	1.75 ± 0.65	1.62		
	RR	1	2.46	2.46		
Insulin sensitivity ($10^{-5} \times$ (min × pmol/l)$^{-1}$)	WW	436	7.30 ± 4.47	6.54	0.083	[c]
	WR	95	6.26 ± 3.48	5.73		
	RR	1	5.20	5.20		
Acute insulin response (pmol × 8 min)	WW	448	2209 ± 1561	1835	0.063	[c]
	WR	98	2432 ± 1608	2125		
	RR	1	4924	4924		
Disposition index	WW	432	13787 ± 9167	11908	0.84	[c]
	WR	95	13029 ± 8051	11671		
	RR	1	25603	25603		
Triglycerides (mmol/l)	WW	1404	2.02 ± 1.79	1.57	0.20	0.40
	WR	267	2.22 ± 1.97	1.67		
	RR	11	1.77 ± 0.74	1.75		
Total cholesterol (mmol/l)	WW	1404	5.58 ± 1.19	5.50	0.23	0.37
	WR	267	5.70 ± 1.31	5.58		
	RR	11	5.38 ± 0.75	5.25		
HDL (mmol/l)	WW	1404	1.18 ± 0.33	1.14	0.038	0.067
	WR	267	1.14 ± 0.33	1.10		
	RR	11	1.20 ± 0.28	1.11		
HDL ratio	WW	1404	0.22 ± 0.07	0.21	0.0073	0.013
	WR	267	0.21 ± 0.07	0.20		
	RR	11	0.23 ± 0.05	0.21		

Table 2. Continued

Outcome	Genotypes	*n*	Mean ± SD	Median	Allelic *p* value[a]	Genotypic *p* value[b]
Mean systolic blood pressure (mm Hg)	WW	1402	143.6 ± 23.1	140.0	0.43	0.31
	WR	263	145.6 ± 25.3	142.0		
	RR	12	134.8 ± 18.7	131.5		
Mean diastolic blood pressure (mm Hg)	WW	1402	82.8 ± 10.8	82.0	0.97	0.95
	WR	263	83.0 ± 11.5	82.0		
	RR	12	81.8 ± 12.2	79.0		

[a] *p* value from the test of statistical significance combining the genotypes WR and RR versus WW.
[b] *p* value from the overall test of statistical significance of the three genotypes.
[c] These genotypic *p* values were not calculated since there was only RR subject

ing numbers for the 219 control subjects who were successfully genotyped were 172 WW, 44 WR, and 3 RR. The allele frequency for the variant was estimated at 0.085 when families were analysed. There were no significant age or sex differences in the distribution of either genotypes or the variant allele (data not shown). A comparison of allele frequencies between affected siblings (allele frequency, $q = 0.08$) and control subjects ($q = 0.11$) was not significant ($p = 0.16$) nor was the corresponding comparison between affected siblings and unaffected spouses ($q = 0.09$; $p = 0.58$). The genotype distributions were consistent with the Hardy-Weinberg equilibrium ($p = 0.76$).

For each of the differences reported previously as significant and under the models assumed, we had excellent statistical power (> 0.99). In addition, we had good power (0.90) to detect relatively small differences in the same traits (Table 1). Since the familial correlations for these traits were modest (within family correlation ranges from 0.001–0.36) these power calculations are expected to be good approximations.

We found no evidence of a difference in the Type II diabetic status rate between family subjects with and without the W64R variant (odds ratio [OR] = 0.86; 95 % confidence internal, CI 0.57–1.29; $p = 0.46$), even after adjusting for age, sex and BMI ($p = 0.58$). Likewise, there was no difference among the three genotypes in diabetic status ($p = 0.70$); the RR genotype to the wild-type genotype OR was 0.71 (95 % CI 0.22–2.32; $p = 0.57$) and the WR to wild-type OR was 0.86 (95 % CI 0.56–1.31; $p = 0.48$). Under a recessive model for disease (RR genotype versus the remaining genotypes) we estimate an OR of 0.76 (95 % CI 0.23–2.46; $p = 0.65$). We found no evidence of an association between the variant and age at diagnosis of Type II diabetes in either the allelic or genotypic models (Table 2).

For each of the anthropometric traits we had substantial power to detect meaningful differences between those with and without the variant (Table 1). Still, we found no significant relation between the variant and any of these measurements (Table 2), even after adjusting for age, sex, diabetic status and smoking.

Similar values were seen in people with and without the variant and among the three genotypes for both the fasting plasma glucose and fasting serum insulin (Table 2). Adjusting for age, sex, Type II diabetic status and BMI did not give rise to a significant association between the variant and fasting insulin or fasting glucose. There was, however, a modest association between the W64R variant and lower fasting insulin concentrations when the analysis was repeated using the general exclusion criteria (see Methods) and adjusting for for age, diabetic status and BMI ($p = 0.034$).

We found no significant association between the W64R variant with any minimal model variables, systolic or diastolic blood pressures, serum triglycerides and serum total cholesterol (Table 2). When diabetic status, age, sex, BMI, smoking status were entered into the model the lack of evidence for association for these traits persisted.

In contrast, HDL values were slightly lower in those with the variant (1.14 ± 0.33 mmol/l) compared with those without (1.18 ± 0.33 mmol/l) ($p = 0.038$) and evidence for this difference was strengthened by adjusting for age, sex, BMI, diabetic status, smoking and alcohol status ($p = 0.005$). In the genotypic analysis RR homozygotes tended to have slightly higher HDL values than the rest of the sample ($p = 0.067$). Similarly, there was an association detected between the variant and evidence for lower HDL ratios in both the allelic ($p = 0.007$) and genotypic ($p = 0.013$) analyses and this difference was strengthened by adjusting for age, sex, BMI, and diabetic status in an allelic analysis ($p = 0.0004$). Heterozygote people tended to have lower HDL ratios than wild-type people whereas RR homozygotes tended to have higher ratios than wild-type subjects (Table 2).

Discussion

Our analysis concentrated on testing associations reported in four previous studies on the W64R variant [12–14, 19]. An association between RR homozygotes and age of onset for Type II diabetes in Pima

Indians had been found previously [14]. Many studies in different ethnic groups followed where the frequency of the W64R variant ranged from 0.04 in Swedes [23] to 0.31 in Pima Indians [14]. No single study has shown a difference in the frequency of the W64R allele in diabetic subjects compared to non-diabetic people although there have been associations with age at onset of diabetes in Pima Indians [14], in Finns [12], and in Japanese [24]. There were two other reports which have, however, shown no association with age at onset of diabetes in independent Japanese [25] and Finnish samples [26].

Moderate associations in Japanese subjects have been found for visceral fat mass after adjusting for covariates [27], for BMI in non-diabetic subjects [28], and for BMI in both diabetic and non-diabetic subjects [24]. In contrast, results from a fourth Japanese study [25], two Caucasian-based studies [23, 26], and a study of Pima Indians [14] showed no associations with obesity-related traits. Following the French study of morbidly obese subjects where there was an association with weight and rate of weight gain [13], two Finnish [12, 26] and one Swedish study [29] failed to replicate the associations in similar Caucasian samples. Furthermore, the presence of the variant was only weakly associated with the insulin resistance in one Finnish study ($p = 0.04$) [12] whereas in another, no association was detected [26].

Taken together, these (and other) conflicting results have brought into question the possible role of the W64R variant in human disease. All associations to date have been modest. Conversely, because so many traits have been analysed, some reported associations are likely to represent false positives. A degree of hypothesis drift has also occurred where some investigators, in an attempt to find an association, have studied physiological and pathological variables of little relevance to the initially reported associations.

Power estimates have been absent in previous publications on the W64R variant with one notable exception [30]. In the study reported here, the large sample size led to excellent power (> 99 %) to detect most trait differences which were reported previously. We find no evidence in the FUSION sample for an association between the W64R variant and Type II diabetes, age at diagnosis of Type II diabetes, measures of obesity, rate of weight gain, or the features of insulin resistance. Since our unaffected sample contains offspring from affected subjects, we recognize that these offspring could be predisposed to diabetes, potentially influencing the traits of interest. Our statistical power is, however, of sufficient magnitude that we should still be able to detect modest differences even in the presence of this potential confounder.

We also saw no differences in allele frequencies between diabetic subjects and elderly control subjects. Furthermore, the OR for the association with Type II diabetes under a recessive mode of inheritance in our data set was 0.76 which is in the opposite direction to results from a recent meta-analysis in which the OR was estimated to be 1.71 (CI = 1.01–2.89), suggesting that RR homozygotes had a higher frequency of diabetes [31].

We did detect a moderate association between the variant and lower fasting insulin concentrations ($p = 0.034$) after adjusting for covariates and excluding all people on prescribed medication expected to affect insulin measurements (including insulin). At least three previous studies have looked at fasting insulin concentrations in a Northern European population. In a Danish study three RR subjects showed a borderline significant association with higher fasting insulin concentrations compared with the wild-type ($p = 0.061$) [19] whereas in similar Finnish samples no differences were detected [12, 26]. The implications of our significant result are not clear, given that the differences between the genotypic-specific means are small and other studies point to an opposite trend, where the variant is associated with slightly higher fasting insulin concentrations.

An association was also found in our study between the W64R variant and the HDL ratio under both allelic ($p = 0.007$) and genotypic analyses ($p = 0.013$). This result is a little paradoxical, however, since heterozygotes have decreased HDL ratios whereas RR homozygotes ($n = 11$) have higher HDL ratios compared with other genotypic groups. Therefore, the W64R variant is not functioning as a predisposing allele in a dose-dependent manner. We believe this result to be of no biological meaning, probably representing a false positive. The other Finnish studies [12, 26, 32] did not find an association with HDL-cholesterol or with the HDL ratio and only one other report in a Japanese cohort [27] found a change in HDL-cholesterol associated with variant homozygotes ($n = 6$). In direct contrast to our findings, these investigators reported a decrease in HDL-cholesterol for RR genotypes compared with wild-type, WW.

Recently, both in vivo and in vitro studies have, in general, shown no functional difference between the variant form of the receptor compared with the wild form [29, 33, 34, 35, 36]. These reports are compatible with the failure to observe phenotypic consequences of the variant in the large data set reported here. Taking all results together, we conclude that the β_3-adrenergic receptor does not have a major role in diabetes and obesity-related traits in our Finnish sample. Family-based studies where there is adequate control for the effects of linked loci and for population stratification could be alternative ways to verify a role of the W64R variant [37, 38].

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