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ARTICLE

Polymorphisms of the gene encoding adiponectin and glycaemic outcome of Chinese subjects with impaired glucose tolerance: a 5-year follow-up study

A. W. K. Tso · P. C. Sham · N. M. S. Wat · A. Xu · B. M. Y. Cheung · R. Rong · C. H. Y. Fong · J. Y. Xu · K. K. Y. Cheng · E. D. Janus · K. S. L. Lam

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Abstract
Aims/hypothesis Polymorphisms of the gene encoding adiponectin (*ADIPOQ*) have previously been associated with type 2 diabetes in Europid and Japanese subjects, but not in Pima Indians. The aim of this study was to determine the contribution made by *ADIPOQ* gene variants to glycaemic status in southern Chinese individuals.
Subjects and methods Sixty unrelated subjects were screened for single-nucleotide polymorphisms (SNPs) in the *ADIPOQ* gene by direct sequencing. The association of tagging SNPs with the outcome of glycaemic status in 262 subjects with impaired glucose tolerance (IGT) was examined in a 5-year prospective study.
Results We identified 15 polymorphisms in the *ADIPOQ* gene, ten of them constituting the tagging SNPs. At 5 years, 39.7% of the subjects with IGT had regressed to NGT, 41.2% had persistent IGT or impaired fasting glucose and 19.1% had developed diabetes. Only the T45G polymorphism was associated with persistent hyperglycaemia at 5 years (p=0.001). Haplotypes formed by the addition of other SNPs, as haplotype blocks or pairs, did not confer greater association than T45G alone. On logistic regression analysis, T45G independently predicted persistent hyperglycaemia at 5 years (OR=2.25, 95% CI 1.29–3.95, G carriers vs TT; p=0.005). It also predicted persistent hyperglycaemia in a nested case–control study involving 158 sex- and age-matched controls with persistent NGT (p=0.012, adjusted for BMI), and that of diabetes or glycaemia progression (p<0.05) in a meta-analysis that also included two published studies in Europid subjects.
Conclusions/interpretation Our findings support a significant role of this common *ADIPOQ* gene polymorphism in predicting glycaemic status in southern Chinese people.

Keywords Adiponectin · Diabetes · Gene · Hyperglycaemia · Impaired glucose tolerance · IGT · Single-nucleotide polymorphism

Abbreviations
OR odds ratio

A. W. K. Tso · N. M. S. Wat · A. Xu · B. M. Y. Cheung · R. Rong · C. H. Y. Fong · J. Y. Xu · K. K. Y. Cheng · K. S. L. Lam (✉)
Department of Medicine, University of Hong Kong,
Queen Mary Hospital,
102 Pokfulam Road,
Pokfulam, Hong Kong
e-mail: ksllam@hkucc.hku.hk

P. C. Sham
Genome Research Centre, University of Hong Kong,
Queen Mary Hospital,
Pokfulam, Hong Kong

A. Xu · B. M. Y. Cheung · K. S. L. Lam
Research Centre of Heart, Brain, Hormone and Healthy Aging,
University of Hong Kong,
Queen Mary Hospital,
Pokfulam, Hong Kong

E. D. Janus
Clinical Biochemistry Unit, University of Hong Kong,
Queen Mary Hospital,
Pokfulam, Hong Kong

Present address:
E. D. Janus
Greater Green Triangle, University Department of Rural Health,
Warrnambool, VIC 3280, Australia

Introduction

Adiponectin is an adipokine with insulin-sensitising properties. In mice, administration of recombinant adiponectin

promotes lipid beta-oxidation in skeletal muscles [1], reduces hepatic gluconeogenesis [2] and improves glucose tolerance and insulin sensitivity [3]. In humans, serum adiponectin level correlates inversely with insulin resistance, hypoadiponectinaemia being found in subjects with obesity or type 2 diabetes mellitus [4].

The gene encoding adiponectin (*ADIPOQ*) is located at chromosome 3q27 [5], a region previously identified in genetic linkage studies to house a diabetes susceptibility locus [6]. Several single-nucleotide polymorphisms (SNPs) and mutations of the *ADIPOQ* gene were shown in Japanese and Europid populations to be associated with diabetes [7–9], obesity [10] or the metabolic syndrome [11]. However, the association of SNPs of the *ADIPOQ* gene with type 2 diabetes and IGT/IFG in various populations has been very inconsistent, different studies finding associations with different SNPs; these results were subsequently replicated in some studies but not others [6–11]. No systematic analysis of the *ADIPOQ* gene with regard to diabetes or IGT has yet been performed in Chinese people.

We examined the *ADIPOQ* gene to identify polymorphisms in a group of southern Chinese people. The effects of these polymorphisms on the glycaemic outcome of a cohort of subjects with IGT were then assessed in a 5-year longitudinal follow-up study.

Subjects and methods

Subjects

An initial pilot study was conducted to identify the polymorphisms in the *ADIPOQ* gene in 60 unrelated Chinese subjects (male/female ratio 37/23; BMI 32±12.6 kg/m^2 [mean±SD]; 72% NGT, 8% IGT, 20% diabetes) recruited from among the hospital staff and patients of the Endocrine Clinic, Queen Mary Hospital, Hong Kong. Blood samples were taken after informed written consent had been given. The patients were referred for obesity and underlying causes had been excluded.

A second cohort, consisting of subjects with IGT, was recruited from the population-based Hong Kong Cardiovascular Risk Factors Prevalence Study [12, 13], in which unrelated Chinese subjects were randomly invited to undergo a comprehensive assessment of cardiovascular risks, including a 75 g OGTT. In 1997, 322 subjects with IGT and 322 sex- and age-matched subjects with NGT were invited to participate in a longitudinal study to assess the natural history of IGT in Chinese people. At baseline, all subjects with IGT were given similar dietary and exercise advice but active medical therapy was not initiated. The subjects returned at 2 and 5 years to repeat the OGTT and were classified as having NGT, IGT, IFG or diabetes according to the diagnostic criteria of the World Health Organization (WHO, 1998) [14]. Those with IGT/IFG/DM at year 5 were collectively defined as having persistent hyperglycaemia. At each visit, body weight, height, BMI, waist circumference and resting blood pressure were measured or calculated as described in published reports of the second-year assessment [12, 13]. Only subjects with IGT whose baseline fasting plasma glucose was below 7 mmol/l (fulfilling the 1998 WHO criteria) and who had consented to genetic analysis were included in this analysis of the 5-year glycaemic outcome (n=262). For the nested case–control study, the controls were subjects with NGT who had normal fasting and 2-h plasma glucose (1998 WHO criteria: <6.1 and<7.8 mmol/l, respectively [14]) at baseline and again at year 5. The study was approved by the Ethics Committee of the Faculty of Medicine, University of Hong Kong.

Fasting glucose, insulin, triglyceride, LDL and HDL were measured as described [12, 13]. Adiponectin was measured for subjects with available stored baseline serum (n=159) using an in-house adiponectin sandwich ELISA assay (inter-assay and intra-assay coefficients of variance of 6.2–8.3% and 5.1–6.4%, respectively) [15].

Genetic analysis

Direct PCR sequencing was used to screen for polymorphisms in the *ADIPOQ* gene. Eleven pairs of primers were constructed to cover 4.6 kb of the gene, and included the promoter region from 1,800 bp upstream of the transcriptional start site and up to +2,018 at the 3′ end. PCR products were sequenced using the ABI Prism DNA sequencing kit (Applied Biosystems, Foster City, CA, USA) on an automated sequencer (ABI Prism 3700 DNA Analyzer; Applied Biosystems). SNPs identified were assigned position labels according to their locations relative to the A of the ATG sequence, the initiator methionine of the *ADIPOQ* gene.

Genotyping for SNPs C-11377G, T45G, G276T and T639C was performed using an RFLP method. SNPs G-12140A, C-11043T, C-10677T and A-3971G were determined by allelic discrimination using Taqman system (Applied Biosystems). The SNPs A-11426G and A-4041C were genotyped using mass spectrometry with the Sequenom platform (Sequenom, San Diego, CA, USA). All primers, restriction enzymes, probes and conditions of the above reactions are available upon request. For quality control of genotyping, the subjects were distributed randomly across plates, and every 96-well plate included two samples with known genotypes detected by direct sequencing, one negative control (water) and five duplicate samples. There were no genotyping errors for positive or

negative control samples; if the consistency of duplicate samples was not greater than 99.5% the plate was considered a failure and was repeated.

Statistical analysis

Linkage disequilibrium between the polymorphisms was analysed using the program Genecounting version 1.80 (http://www.smd.qmul.ac.uk/statgen/dcurtis/software.html, last accessed in May 2006).

Currently, data on linkage disequilibrium of the adiponectin gene in Chinese people are not comprehensive in the public domain, even after completion of the HapMap Project (http://www.hapmap.org, last accessed on 1 March 2006). Tagging SNPs of the gene for further analysis were therefore selected on the basis of our own data set from the pilot study; these consisted of all SNPs having low linkage disequilibrium with other polymorphisms (r^2<0.8), and a representative SNP from each group of polymorphisms in tight linkage disequilibrium (r^2>0.8). Haplotype frequencies were estimated using Genecounting and those higher than 1% were further analysed using Genecounting and the WHAP program, version 2.05 (available at http://pngu.mgh.harvard.edu/~purcell, last accessed in May 2006) for association with year 5 glycaemic status.

The association of baseline demographic, biochemical and genotypic variables with glycaemia was tested using ANOVA or the χ^2 test, as appropriate. Variables with skewed distribution were logarithmically transformed prior to analysis. Correction for multiple testing was made using Bonferroni's correction. Variables showing p<0.1 were tested for correlation prior to analysis with backward logistic regression to determine the independent baseline predictors of year 5 glycaemic status. The association of genotype distribution and haplotype frequencies with year 5 glycaemic status was examined with the χ^2 test, and the likelihood ratio test using the WHAP program. For SNPs with low frequency of the variant allele, subjects homozygous and heterozygous for the variant allele were grouped together for analyses. The IGT cohort sample had more than 80% power to detect an odds ratio of greater than 2 between the G+ genotype (where + denotes G or T) and persistence of hyperglycaemia when the prevalence of persistent hyperglycaemia within the cohort was 60%. Statistical significance was indicated by p values less than 0.05.

In performing the meta-analysis, the data of each study were summarised in the form of 2×2 tables, giving the subject counts in different combinations of genotype (TT vs G+) and outcome (diabetes vs no diabetes, and progression vs non-progression). The logged odds ratio and its sampling variance were calculated for each table using a standard formula. The logged odds ratios were combined across the three studies, inversely weighted by their variances. This produced a combined logged odds ratio and its sampling variance, which were then converted into a pooled odds ratio with its 95% confidence interval, and a χ^2 statistic with a p value. The results obtained were further confirmed using the Mantel–Haenszel method.

Results

In the analysis of the *ADIPOQ* gene in the pilot study, we identified 15 polymorphisms in our Chinese subjects (Fig. 1). Of these, A-11426G and -11156 insCA were in complete linkage disequilibrium (r^2=1). Strong linkage disequilibrium (r^2>0.9) was also present between T45G, A349G and G967A, and between T639C, A712G and 2019del, as shown in Fig. 1b. SNP G-11391A, previously reported in Europid groups, was not found in our cohort. Ten tagging SNPs were thus selected for further study, six of which (rs1681194, rs266729, rs822396, rs2241767, rs3821799, rs3774262) were also tagging SNPs in the HapMap for northern Chinese subjects. Five of the 15 polymorphisms we identified were not listed in the HapMap for Han Chinese in Beijing (northern Chinese): all except G276T (intron 2; rs1501299) were tagged by other SNPs typed in the HapMap (r^2>0.5 for all four, and >0.9 for two). On the other hand, there were 16 SNPs typed in the HapMap Chinese samples but not in our cohort: ten of these 16 were tagged by at least one of the six selected HapMap tagging SNPs at an r^2 threshold of 0.8, and another three at a threshold of 0.5. Of the remaining three, two were rare SNPs with allele frequencies of 2.9 and 1.1%, respectively, and the third, rs12495941 (G-2668T), was located in intron 1, 2,660 bp from the splice acceptor site, and was not involved in any transcription binding site, based on the Match program (http://www.gene-regulation.com, last accessed on 1 March 2006).

The associations of our ten tagging SNPs with the year 5 glycaemic outcome of a cohort of IGT subjects (n=262) were then examined in a prospective follow-up study. The genotype data of the SNPs did not deviate significantly from Hardy–Weinberg equilibrium.

In the longitudinal study, 39.7% of the 262 IGT subjects had reverted at year 5 to normoglycaemia (NGT), 41.2% had IGT or IFG, and 19.1% had progressed to diabetes mellitus. Subjects with persistent hyperglycaemia (IGT/IFG/diabetes) had more adverse risk factors at baseline (Table 1). After correction for multiple testing, male gender, high baseline BMI, waist circumference, triglyceride, fasting and 2-h post-OGTT glucose and low HDL were significantly associated with persistent hyperglycaemia at year 5.

Table 2 shows the genotype distribution with respect to the year 5 glycaemic status. Only the T45G polymorphism was significantly associated with the year 5 glycaemic

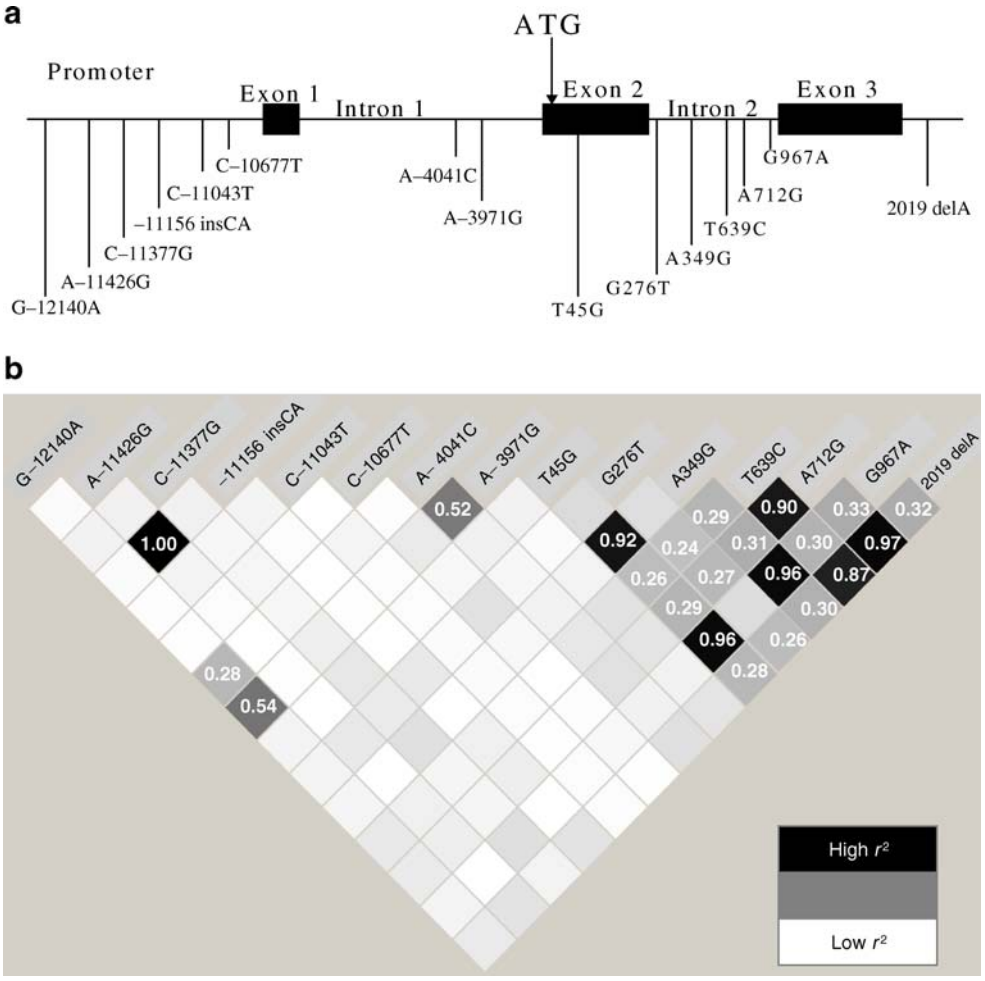

Fig. 1 Diagrams illustrating **a** the positions and **b** the linkage disequilibrium coefficients (r^2) of the polymorphisms of the *ADIPOQ* gene identified in 60 unrelated southern Chinese subjects. Values represent r^2 between SNPs; for cells without numbers, r^2<0.2

status (p=0.001), which remained significant after correction for the testing of ten tagging SNPs. The risk of persistent hyperglycaemia at year 5 was higher in subjects carrying the G allele (GG or GT), with an odds ratio of 2.34 (95% CI 1.41–3.90) compared with TT (p=0.001).

Subjects carrying the T45G G allele had more adverse risk factors at baseline (Table 3), including higher waist circumference, higher triglyceride level and lower HDL level. The adiponectin level, measured in a subgroup, was lower in G carriers and more evident in male subjects

Table 1 Baseline clinical characteristics of the subjects according to the year 5 glycaemic status

	NGT (n=104)	IGT/IFG (n=108)	Diabetes mellitus (n=50)	p value
Age (years)	48.7±12.1	52.0±11.0	50.5±13.3	NS
Sex (M/F)	32/72	52/56	24/26	0.026
BMI (kg/m^2)	24.5±3.4	25.9±3.5	27.3±3.1	<0.001
Waist circumference (cm)	M, 86.2±8.1	M, 88.2±9.1	M, 87.8±7.5	NS
	F, 76.3±8.7	F, 80.4±8.2	F, 84.4±8.9	<0.001
Hypertension (%)	37.5	43.5	52.0	NS
Fasting glucose (mmol/l)	5.2±0.5	5.4±0.5	5.6±0.5	<0.001
2-h Glucose (mmol/l)	8.6±0.6	9.0±0.9	9.4±0.8	<0.001
Fasting insulin (mIU/l)[a]	6.4 (3.7–9.4)	6.7 (4.2–9.6)	7.0 (5.9–8.4)	0.060
Triglyceride (mmol/l)[a]	1.0 (0.7–1.5)	1.3 (1.0–1.9)	1.4 (1.0–1.8)	0.001
HDL (mmol/l)	1.23±0.33	1.18±0.03	1.12±0.28	0.001

[a]Analysis was performed after logarithmic transformation of respective parameters; values are presented as medians (interquartile range)
F Female, *M* male, *NS* not significant

Table 2 Genotype distribution and allele frequencies of *ADIPOQ* SNPs according to year 5 glycaemic status

Polymorphism	Genotype	Glycaemic status at 5 years (%)		*p* value
		Normoglycaemia	Hyperglycaemia	
G-12140A[a]	GG	67.0	77.7	
	GA	32.0	22.3	0.056
	AA	1.0	0	
	A allele (%)	17.0	11.1	
A-11426G[a]	AA	77.0	74.0	
	AG	18.8	23.3	0.585
	GG	4.2	2.7	
	G allele (%)	13.5	14.3	
C-11377G	CC	50.0	54.6	
	CG	41.8	38.8	0.477
	GG	8.2	6.6	
	G allele (%)	29.1	26.0	
C-10677T	CC	86.1	89.7	
	CT	13.9	10.3	0.381
	T allele (%)	7.0	5.0	
C-11043T[a]	CC	92.3	94.9	
	CT	7.7	4.4	0.385
	TT	0	0.7	
	T allele (%)	3.8	2.8	
A-4041C	AA	66.7	74.0	
	AC	31.3	25.3	0.217
	CC	2.1	0.7	
	C allele (%)	17.7	15.4	
A-3971G[a]	AA	75.0	77.2	
	AG	24.0	22.8	0.680
	GG	1.0	0	
	G allele (%)	13.0	11.4	0.001
T45G	TT	64.8	43.9	
	TG	27.6	49.7	0.001
	GG	7.6	6.4	
	G allele (%)	21.4	31.2	
G276T	GG	47.1	51.9	
	GT	38.5	37.0	0.638
	TT	14.4	11.0	
	T allele (%)	33.7	29.5	
T639C	TT	32.4	34.7	
	TC	51.0	50.0	0.916
	CC	16.7	15.3	
	C allele (%)	42.2	40.3	

[a]Subjects homozygous for the variant allele were grouped together with heterozygous subjects for analysis

(p=0.044). Baseline fasting glucose and 2-h post-OGTT glucose were similar between TT and G carriers. There was no significant difference in the weight change from baseline between G carriers and TT subjects (0.18±3.44 vs 0.22± 3.77 kg; not significant).

Backward logistic regression analysis was performed to determine the significant factors contributing to glycaemic status at year 5. The model included age, sex, waist circumference, 2-h post-OGTT glucose load, HDL and T45G genotype. The presence of the G allele of T45G was a significant independent predictor of persistent hyperglycaemia (odds ratio [OR]=2.25, 95% CI 1.29–3.95, G+ vs TT; p=0.005), together with waist circumference (OR=1.04, p=0.025) and 2-h post-OGTT glucose (OR=2.30; p<0.001). Similar significance for the G allele was obtained (OR=2.19; p=0.005) if fasting glucose replaced 2-h post-OGTT glucose in the model. Inclusion of weight change over 5 years in the model did not affect the overall finding; the odds ratio of G+ for persistent hyperglycaemia remained at 2.25 (95% CI 1.35–3.75; p=0.002).

Table 3 Baseline clinical characteristics of the subjects according to T45G genotype

	T/T (*n*=137)	G/G or G/T (*n*=125)	*p* value
Age (years)	49.2±11.6	51.9±12.3	0.064
Sex (M/F)	49/88	59/66	0.078
BMI (kg/m^2)	25.3±3.4	25.8±3.6	NS
Waist circumference (cm)	M, 87.6±8.0	M, 87.3±8.8	NS
	F, 77.9±8.7	F, 80.9±9.2	0.038
Hypertension (%)	39.4	46.4	NS
Fasting glucose (mmol/l)	5.4±0.6	5.4±0.5	NS
2-h Glucose (mmol/l)	8.9±0.8	8.9±0.9	NS
Fasting insulin (mIU/l)[a]	6.0 (3.7–8.6)	6.8 (4.3–9.1)	0.072
Triglyceride (mmol/l)[a]	1.1 (0.8–1.7)	1.4 (1.0–1.9)	0.008
HDL (mmol/l)	1.26±0.34	1.15±0.28	0.007
Adiponectin (μg/ml), *N*=159	*n*=81	*n*=78	
	5.4 (4.0–7.5)	4.5 (2.8–6.6)	0.061
	M, 5.3 (3.2–7.2)	M, 4.5 (2.8–6.6)	0.044
	F, 5.4 (4.1–7.6)	F, 5.2 (3.5–7.2)	NS

[a]Analysis was performed after logarithmic transformation of respective parameters; values are presented as medians (interquartile range)
F Female, *M* male, *NS* not significant

Haplotype analyses showed that only paired haplotypes which included T45G showed a significant effect on glycaemic status (T45G with G-12140A, C-10677T, A-4041C, G276T or T639C, $p<0.05$). Further analysis using the likelihood ratio test of the WHAP program confirmed that no other SNPs contributed an additional gene effect above that of T45G. A significant protective effect of a haplotype block including 45T was found (Table 4), but associations of haplotypes with low frequencies need to be interpreted with caution.

The presence of T45G (G+ vs TT) was associated with an odds ratio of 1.23 (not significant) for the progression to diabetes in our 262 IGT subjects. Figure 2 summarises the findings of a meta-analysis which included, apart from this study (Chinese IGT), data from two recently published prospective studies in Europids, the STOP-NIDDM trial [16] and the DESIR study [17]. In the former study [16], among IGT subjects on placebo, 47 of 81 G carriers progressed to diabetes, compared with 143 of 333 in the TT group. In the latter study [17], seven of 903G carriers

Table 4 *ADIPOQ* haplotype frequencies and association analysis with year 5 glycaemic status

G-12140A	A-11426G	C-11377G	C-11043T	C-10677T	A-4041C	A-3971G	T45G	G276T	T639C	Haplotype frequency		
Alleles										IGT/diabetes	NGT	*p* value
G	A	C	C	C	A	A	G	G	T	0.232	0.169	0.082
G	A	G	C	C	A	A	T	G	C	0.147	0.119	NS
G	A	C	C	C	A	A	T	G	C	0.102	0.085	NS
G	A	G	C	C	A	A	T	T	T	0.080	0.102	NS
A	A	C	C	C	C	G	T	G	C	0.079	0.097	NS
G	G	C	C	C	A	A	T	T	T	0.082	0.075	NS
G	A	C	C	C	A	A	T	T	T	0.032	0.059	NS
G	A	C	C	T	A	A	T	T	T	0.030	0.031	NS
G	A	C	C	C	A	A	T	G	T	0.028	0.042	NS
G	G	C	C	C	A	A	G	G	T	0.023	0.026	NS
G	G	C	C	C	A	A	T	G	C	0.018	0.026	NS
G	A	C	C	C	A	A	G	T	T	0.024	0.000	NS
G	A	C	C	T	C	G	T	G	C	0.016	0.009	NS
A	A	G	C	C	A	A	T	G	C	0.005	0.028	0.026
G	G	C	C	C	A	A	G	T	T	0.017	0.000	NS

Haplotype frequencies were estimated using the Genecounting program, and haplotype association analysis was performed with the WHAP program
Only haplotypes with frequencies >0.01 are shown

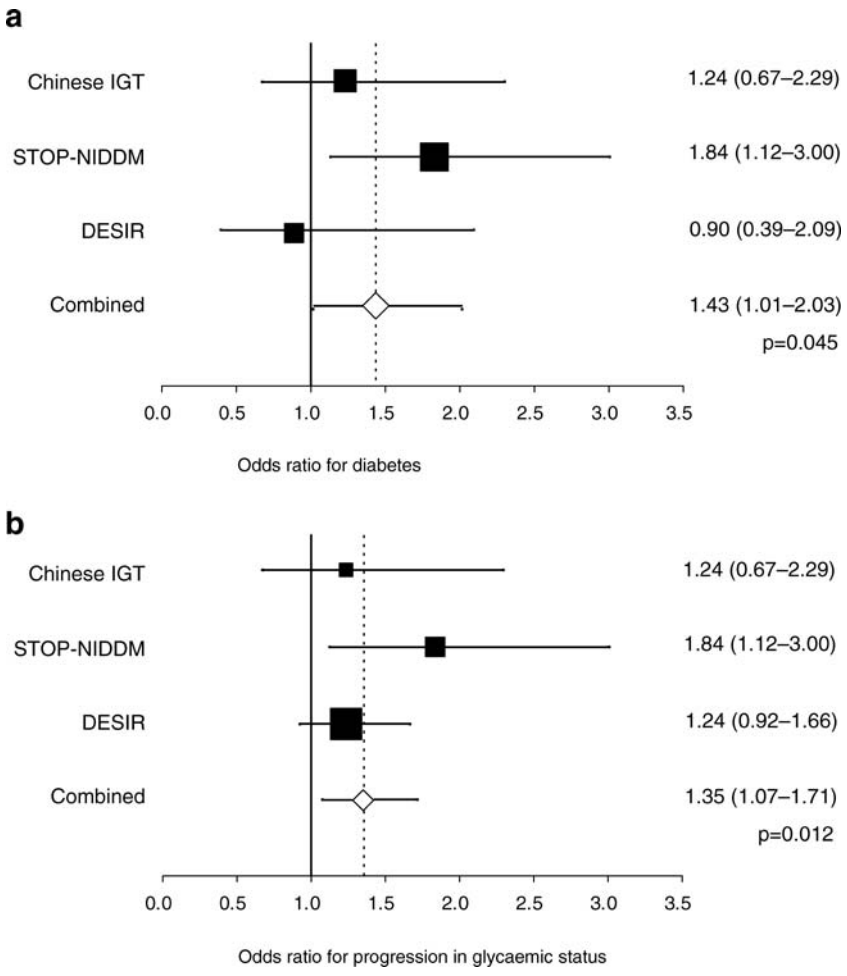

Fig. 2 Meta-analysis of three prospective studies (this study and [16] and [17]) on the association of the *ADIPOQ* T45G polymorphism with **a** development of diabetes mellitus and **b** progression in glycaemic status, on long-term follow-up

with normal fasting glucose (NFG) developed diabetes, compared with 24 of 2,816 NFG subjects with the TT genotype. On the other hand, 65 of the 903 G carriers progressed from NFG to IFG or diabetes, compared with 164 of 2,980 in the TT group [17]. The combined odds ratio for the three studies was 1.43 for the development of diabetes (95% CI 1.01–2.03; p=0.045; Fig. 2a) and 1.35 (95% CI 1.07–1.71; p=0.012; Fig. 2b) for progression in glycaemic status. The results remained essentially the same when the Mantel–Haenszel method was used.

The effect of T45G on persistent hyperglycaemia was further tested in a nested case–control study. The cases were the 158 subjects with IGT who remained with IGT or developed diabetes by year 5. The controls consisted of 158 sex- and age-matched NGT subjects who remained with NGT by year 5. The baseline characteristics of the two groups are summarised in Table 5. The presence of the G allele was significantly associated with persistent hyperglycaemia (OR=1.85, 95% CI 1.18–2.89, G+ vs TT; p=0.007). This association remained significant after correcting for waist circumference (OR=1.73, 95% CI 1.09–2.75, G+ vs TT; p=0.021), BMI (OR=1.81, 95% CI 1.14–2.88, G+ vs TT; p=0.012) or weight change over 5 years (OR=1.84, 95% CI 1.18–2.88, G+ vs TT; p=0.007).

In this nested case–control study, a low serum adiponectin concentration was significantly associated with persistent hyperglycaemia. Subjects with serum adiponectin levels in the lowest tertile (<3.8 μg/ml in men and <5.0 μg/ml in women) had an odds ratio of 3.55 (95% CI 1.91–6.62; p<0.001) for persistent hyperglycaemia compared with those with levels in the highest tertile (≥6.1 μg/ml in men and ≥7.6 μg/ml in women), and an odds ratio of 2.25 (95% CI 1.23–4.13; p=0.008) compared with those with levels in the second tertile (3.8–6.1 μg/ml in men and 5.0–7.6 μg/ml in women). The increased risk of persistent hyperglycaemia for subjects with serum adiponectin in the lowest tertile remained significant after correcting for waist circumference (OR=2.92; p=0.001; OR=2.21; p=0.013, respectively) or BMI (OR=2.92; p=0.001; and OR=2.05; p=0.025, respectively).

Table 5 Baseline characteristics of subjects with persistent normoglycaemia (NGT-NGT) and subjects with persistent hyperglycaemia (IGT-IGT/IFG/DM)

	NGT-NGT (*n*=158)	IGT-IGT/IFG/DM (*n*=158)	*p* value
Sex (M/F)	75/83	75/83	1.000
Age (years)	51.2±12.4	51.8±11.8	0.649
Waist circumference (cm)	M, 83.0±8.9	M, 88.0±8.6	0.001
	F, 75.8±9.0	F, 81.7±8.6	<0.001
BMI (kg/m^2)	24.4±3.7	26.3±3.4	<0.001
Serum adiponectin (μg/ml)	M, 5.8 (3.8–7.2)	M, 5.0 (5.1–7.5)	0.013[a]
	F, 7.0 (5.1–10.2)	F, 5.1 (3.9–7.4)	<0.001[a]
			<0.001[b]
T45G (G+/TT)	64/94	88/70	0.007

Data are means±SD except for adiponectin (median and interquartile range)
[a]Independent *t*-test on log-transformed adiponectin level
[b]Logistic regression: sex-adjusted log-transformed adiponectin level (*n*=265)
DM Diabetes, *F* female, *M* male

Discussion

In this study, we performed a systematic analysis of the gene encoding adiponectin (*ADIPOQ*) in 60 unrelated Chinese subjects and found 15 polymorphisms, of which ten SNPs were identified as the tagging SNPs in our Chinese population. Subsequent analysis of these tagging SNPs in the longitudinal study revealed, however, that only the T45G polymorphism was a significant predictor of glycaemic status in the Chinese population. The presence of the T45G polymorphism in the *ADIPOQ* gene was an independent risk factor for persistent hyperglycaemia, over a period of 5 years, in Chinese subjects with IGT at baseline, independent of obesity indices.

Previous case–control studies have reported conflicting findings regarding the association of the adiponectin T45G polymorphism with hyperglycaemia. An increased risk of diabetes mellitus was shown in subjects with the GG genotype (OR=1.70) in a Japanese population [7], and the G allele was also associated with reduced insulin sensitivity and increased BMI in a German cohort [10]. However, another group found that the T allele contributed to the risk of hyperglycaemia [11], and others failed to find any association of the T45G polymorphism with diabetes mellitus in Europids [8, 9, 18] and Pima Indians [19]. Most of the conflicting results among reported case–control studies could have been caused by statistical fluctuations, such as sampling errors related to small sample sizes, and reporting biases consequent to inadequate allowance for type 1 error and multiple testing. Other contributing factors may include ethnic differences [7, 8, 19] and differences in inclusion criteria (especially age, sex [18], family history of diabetes and obesity [8, 10, 19]) and diagnostic criteria for diabetes vs non-diabetic controls.

While large case–control studies are more useful for the initial identification of susceptibility SNPs, prospective studies are likely to be better for quantifying the true risk of established variants, compared with cross-sectional studies. The findings of our study were consistent with those of the STOP-NIDDM trial [16] and the DESIR study [17], the only two reported prospective cohort studies addressing the relationship between *ADIPOQ* gene variants and glycaemic progression in both genders. In the former, a 1.8-fold increased risk of progressing from IGT to diabetes (WHO 1985 diagnostic criteria), after a mean follow-up of 3.3 years, was seen in G carriers in the placebo group [16]. In the latter, more NFG subjects with the T45G GG genotype developed hyperglycaemia (IFG or diabetes; American Diabetes Association 1997 criteria) in 3 years (OR=2.71 vs TT) [17]. The risk of persistent hyperglycaemia associated with the presence of the G allele in our IGT subjects was further supported by the results of the nested case–control analysis. Our apparent failure to demonstrate an increased risk in the GG subjects may be attributed to its low genotype frequency, such that only ten of the 158 subjects with persistent hyperglycaemia were homozygous GG. A meta-analysis of these three prospective cohort studies showed that carriers of the G allele had a significantly increased risk of developing diabetes, or progression in hyperglycaemia, on long-term follow-up.

For the other SNPs studied, we found no association with hyperglycaemia, in contrast to previous reports on SNPs A-11426G [9], C-11377G [8, 9] and G276T [7, 11]. This may again be explained by differences in ethnicity and study design. SNP G-11391A, which was previously reported to be associated with diabetes mellitus [8, 17] in both case–control and longitudinal studies, was not found in our study population.

The nature of the risk conferred by adiponectin T45G is unclear. T45G is a silent polymorphism with no amino acid change. However, it has been shown to influence the allele-specific differential expression of the adiponectin gene in

human omental adipose tissue [20]. Previous studies have reported increased [8], decreased [21] or unchanged [18] levels of adiponectin in subjects with the T45G variant. In our study, a lower adiponectin level was found in the G carriers, but the result was only significant in the male subjects. Our sample size did not provide sufficient power to determine whether the association of T45G with the development of diabetes could indeed be explained by its influence on adiponectin expression. It is likely that the effect of the polymorphism is mediated through tight linkage disequilibrium to another, as yet unidentified, functional SNP in the promoter or 3′-untranslated region. Alternatively, it may be acting in concert with another related gene, such as the peroxisome proliferator-activated receptor (PPAR) γ gene, whose Pro12Ala polymorphism has been reported to interact with the adiponectin T45G polymorphism [22].

Association of the T45G polymorphism with obesity has been reported [10, 11], and in the DESIR study the GG group gained more weight after 3 years [17]. In our cohort, the G carriers did not have greater weight gain than the TT subjects. Although they had higher baseline waist circumference, the effect of the gene on hyperglycaemia was independent of baseline BMI or waist circumference in logistic regression, and the propensity to hyperglycaemia in the G carriers could not be explained by weight changes over the 5-year period.

We report the effect of the *ADIPOQ* T45G polymorphism on the reversion to normoglycaemia in high-risk subjects with baseline IGT. IGT is known to be a dynamic state. In the Diabetes Prevention Program, reversion to normoglycaemia occurred in almost 20% of IGT subjects even without lifestyle intervention [23]. Our regression rate of 39.7% was comparable to that reported for Japanese (33.9% in the absence of lifestyle intervention) [24], but it remains possible that the phenomenon of regression to the mean might contribute to the regression rate [25]. Even though we depended on a single OGTT at each time point to determine the glycaemic status, the change in glycaemic status was probably genuine as the risk of persistent hyperglycaemia was also predicted by well-accepted baseline risk factors, such as high BMI and fasting glucose.

In conclusion, subjects carrying the G allele of the *ADIPOQ* T45G polymorphism are at risk of persistent hyperglycaemia on 5-year follow-up in our cohort of Chinese subjects with IGT. A complex interplay between genetic and environmental factors is likely to influence the progression or regression of IGT, and we have shown that the *ADIPOQ* T45G polymorphism may be important in the Chinese population.

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