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Increased risk of obesity associated with the variant allele of the PPARGC1A Gly482Ser polymorphism in physically inactive elderly men

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Abstract *Aims/hypothesis:* The variant allele of the Gly482Ser polymorphism in peroxisome proliferator-activated receptor- γ co-activator-1 α (PPARGC1A or PGC1 α), a critical determinant of skeletal muscle metabolism, has been associated with obesity and type 2 diabetes. Previous studies indicate that these risks depend on sex and environmental triggers such as age. The aim of the present study was to investigate the possible interactions between genotype and age and physical activity on risk of obesity. *Methods:* We genotyped PPARGC1A Gly482Ser, in a population-based study comprising 899 women and 902 men aged between 30 and 75 years in Vara, Sweden. *Results:* Genotyping revealed that 56% of the males and 57% of the females carried the PPARGC1A 482Ser variant allele. Elderly males (≥ 50 years) carrying 482Ser had an increased risk of obesity compared with subjects who were homozygous for the wild-type allele (odds ratio [OR]=1.99, 95% CI 1.14–3.47, $p=0.015$). The risk was restricted to males with a low leisure-time physical activity level, and was significantly weaker (OR=0.44, 95% CI 0.22–0.87, $p=0.018$) for the homozygous 482Gly carriers among this subgroup. No association with obesity was found in elderly males with a high level of physical activity, in younger males, or in females of any age group or level of physical activity. *Conclusions/interpretation:* Our findings confirm that sex and age should be considered

when investigating the influence of the PPARGC1A Gly482Ser polymorphism on metabolic disease. The risk of obesity associated with 482Ser is evident only in physically inactive elderly male subjects. Whenever possible, the level of physical activity should be addressed in future studies on disease risk associated with PPARGC1A Gly482Ser.

Keywords Epidemiological study · Genetics · Obesity · PGC1 α · Physical activity · PPARGC1A Gly482Ser

Abbreviations LTPA: leisure-time physical activity · OR: odds ratio · PPARGC1A: peroxisome proliferator-activated receptor- γ co-activator 1 α

Introduction

Obesity is an important predictor and cause of type 2 diabetes and cardiovascular disease. Both lifestyle factors, such as energy expenditure associated with physical activity, and genetic factors play important roles in the development of obesity. The peroxisome proliferator-activated receptor- γ co-activator 1 α (PPARGC1A, sometimes referred to as PGC1 α), a master regulator of skeletal muscle metabolism, has recently been implicated in obesity and type 2 diabetes by epidemiological and mechanistic studies [1]. The expression of the gene encoding PPARGC1A (PPARGC1A) and a set of genes involved in oxidative phosphorylation is decreased in skeletal muscle from subjects with impaired glucose homeostasis or a family history of type 2 diabetes [2, 3]. These data suggest that PPARGC1A dysfunction is an important early and inherited trait of metabolic disease. A genome scan of obesity has shown a linkage peak in the region that contains PPARGC1A [4]. Importantly, the PPARGC1A Gly482Ser polymorphism has been associated with type 2 diabetes, obesity, and with an age-dependent decrease in PPARGC1A mRNA and levels of the protein [5–8]. We hypothesised that the risk associated with 482Ser is subject to sex- and age-specific effects and may be modulated by physical

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activity. We tested this hypothesis by investigating the influence of the PPARGC1A Gly482Ser polymorphism on obesity in a population-based study with available data on physical activity.

Subjects and methods

Subjects

This study is based on data from a population-based study in Vara, a small community in a rural area of south-western Sweden, as part of the Skaraborg Project. The parameters assessed have previously been described in detail [9]. Inclusion criteria were attendance at an outpatient department for assessment of clinical parameters, completion of health questionnaires, and successful genotyping of the PPARGC1A Gly482Ser polymorphism. A total of 1,811 subjects were surveyed in this study. Of these, 899 females and 902 males had the full information required for the study (Table 1). The participants answered structured questionnaires concerning ethnicity, demography, socioeconomic status, education, lifestyle and mental health. Weight and height were recorded with subjects in light clothing without shoes to the closest 0.1 kg and 1 cm, respectively, and BMI (kg/m^2) calculated. Obesity was defined as a BMI $\geq 30 \text{ kg}/\text{m}^2$. Leisure-time physical activity (LTPA) was divided into four different categories, ranging from sedentary to hard exercise. Subjects who participated in regular physical activity for at least 2 h a week were considered to be physically active during leisure time (level 3 or 4), and those reporting less were considered to have a low physical activity (level 1 or 2) [10]. The research ethical committee in Gothenburg approved the study and informed written consent was obtained from the subjects prior to their participation.

DNA extraction and genotyping

DNA was extracted from whole blood using the QiaGen MiniPrep Kit (Qiagen, Hilden, Germany) at the DNA/RNA Genotyping Laboratory at the SWEGENE Resource Center for Profiling Polygenic Disease (Malmö University Hospital, Lund University, Malmö, Sweden). The PPARGC1A Gly482Ser polymorphism was genotyped using an allelic discrimination assay, which was performed with an ABI 7900 system according to manufacturer's recommendations (Applied Biosystems, Foster City, CA, USA) using the PCR primers 5'-TGGAGAATTGTTCTTACTGAAATCACTGT-3' (forward) and 5'-GGTCATCCCAGTCAAGCTGTTTT-3' (reverse), and the TaqMan MGB probes Fam-5'-ACAAGACCAGTGAAGT-3' and Vic-5'-CAAGACCGGTGAAGT-3'. Genotyping efficiency was 99.8%, and 100% genotyping accuracy was shown when 5% of the samples were re-run. The frequency distribution was in Hardy-Weinberg equilibrium (data not shown).

Statistical analysis

Standard methods were used for descriptive statistics. All analyses were adjusted for age and stratified by sex. Although age was used as a continuous variable, for stratification, subjects were categorised as being 30 to <50 years or ≥ 50 years. Proportions were age-standardised by 5-year age groups, using the whole Vara population as standards. Associations between obesity and categorical variables were analysed by logistic regression and expressed as odds ratios (OR) with 95% CIs. All tests were two-sided, and statistical significance was assumed when the p value was less than 0.05. All statistical analyses were performed using SPSS for Windows XP, version 11.5 (SPSS, Chicago, IL, USA).

Table 1 Characteristics of the study population

Variable	Men		Women	
	<i>n</i> =902		<i>n</i> =899	
	Mean	(SD)	Mean	(SD)
Age (years)	48.0	(11.8)	48.1	(11.9)
BMI (kg/m^2)	27.0	(4.6)	26.8	(4.6)
Waist circumference (cm)	95	(12.0)	85	(11.9)
WHR (cm)	0.94	(0.06)	0.83	(0.06)
Systolic blood pressure (mmHg)	124	(13.8)	120	(13.9)
Diastolic blood pressure (mmHg)	72	(9.3)	69	(9.3)
	<i>n</i>	(%)	<i>n</i>	(%)
Obesity (BMI $\geq 30 \text{ kg}/\text{m}^2$)	172	(20.1)	206	(25.5)
Low LTPA	552	(63.2)	630	(73.2)
PPARGC1A Gly482Ser genotype:				
Gly/Gly	393	(44)	389	(43)
Gly/Ser	408	(45)	406	(45)
Ser/Ser	101	(11)	104	(12)

Means were adjusted for age. Percentages were standardised for age using the population in Vara as the standard. Missing data: waist circumference, five women; WHR, two men, seven women; LTPA, 12 men, eight women

Results

Clinical characteristics and PPARGC1A Gly482Ser genotype distribution are shown in Table 1. Both the men and women were generally overweight, with a mean BMI ($\pm$ SD) of 27.0 ± 4.6 and 26.8 ± 4.6 kg/m², respectively. According to the criteria set out by the World Health Organization, 72% of the men and 60% of the women were overweight or obese, and 20 and 25%, respectively, were obese. Overall, we found no association between 482Ser and obesity in either sex, although a trend for increased risk was noted in the men (OR=1.37, 95% CI 0.97–1.92, $p=0.074$).

When we stratified the population according to age and sex to test our primary hypothesis we observed a significant association between the Ser allele and obesity (OR=1.99, 95% CI 1.14–3.47, $p=0.015$) in elderly males (age ≥ 50 years). When a potential dominant effect of the Ser allele was explored in this subgroup, the calculated odds ratio was 2.09 (95% CI 1.02–4.31) in those who were heterozygous (Gly/Ser) and 3.12 (95% CI 1.14–8.55) in subjects homozygous for the Ser allele (Table 2). Accordingly, upon formal testing, the interaction between PPARGC1A Gly482Ser and age was significant in men ($p=0.025$) but not in women ($p=0.407$). The risk was restricted to males with a low LTPA level; however, the association with obesity was significantly weaker for homozygous 482Gly carriers (OR=0.44, 95% CI 0.22–

0.87, $p=0.018$). The association was absent in younger men and in women of all ages. A low level of LTPA was associated with an increased risk of obesity in both men (OR=2.10, 95% CI 1.43–3.08, $p<0.001$) and women (OR=2.72, 95% CI 1.79–4.15, $p<0.001$). There was no significant interaction between PPARGC1A Gly482Ser and LTPA either in the study population as a whole, or in men or women when analysed separately (data not shown).

Discussion

To our knowledge, this is the first population-based study that has observed an interaction between the PPARGC1A Gly482Ser polymorphism and physical activity with respect to the risk of obesity. Our data confirm that 482Ser is associated with obesity but that this risk is dependent upon age, sex and physical activity. An increased risk was only seen in physically inactive men aged ≥ 50 years. In other words, elderly male homozygous carriers of 482Gly who are physically inactive exhibit relative protection against obesity. Conversely, male carriers of the risk allele may be able to compensate for this risk by means of increased physical activity.

Based on previous information, our a-priori-defined hypothesis was that the risk of obesity associated with 482Ser is influenced by age, sex and level of physical activity [1–8]. The data were primarily analysed in separate

Table 2 The interaction of the PPARGC1A Gly482Ser polymorphism with LTPA and age in the association with obesity in men and women

		Gly/Gly (<i>n</i> =393)		Gly/Ser (<i>n</i> =408)				Ser/Ser (<i>n</i> =101)			
		<i>n</i>	OR	<i>n</i>	OR	95% CI	<i>p</i> value	<i>n</i>	OR	95% CI	<i>p</i> value
Men											
All men		393	1.0	408	1.39	0.97–1.99	0.071	101	1.27	0.73–2.22	0.403
<50 years		249	1.0	262	1.13	0.71–1.78	0.606	72	0.89	0.43–1.84	0.751
≥ 50 years		144	1.0	146	1.92	1.08–3.43	0.027	29	2.36	0.95–5.84	0.064
Low LTPA		233	1.0	248	1.28	0.83–1.98	0.260	71	1.46	0.79–2.70	0.230
High LTPA		154	1.0	155	1.88	0.95–3.73	0.070	29	N/A		
<50 years	Low LTPA	144	1.0	156	0.95	0.55–1.64	0.843	48	0.93	0.42–2.06	0.850
<50 years	High LTPA	101	1.0	103	1.70	0.69–4.20	0.246	23	N/A		
≥ 50 years	Low LTPA	89	1.0	92	2.09	1.02–4.31	0.045	23	3.12	1.14–8.55	0.027
≥ 50 years	High LTPA	53	1.0	52	2.37	0.81–6.90	0.114	6	N/A		
		Gly/Gly (<i>n</i> =389)		Gly/Ser (<i>n</i> =406)				Ser/Ser (<i>n</i> =104)			
		<i>n</i>	OR	<i>n</i>	OR	95% CI	<i>p</i> value	<i>n</i>	OR	95% CI	<i>p</i> value
Women											
All women		389	1.0	406	1.07	0.76–1.49	0.706	104	1.15	0.69–1.93	0.593
<50 years		249	1.0	261	1.00	0.63–1.57	0.989	67	1.10	0.55–2.18	0.791
≥ 50 years		140	1.0	145	1.16	0.70–1.92	0.573	37	1.20	0.55–2.62	0.654
Low LTPA		270	1.0	288	1.16	0.79–1.70	0.442	72	1.46	0.82–2.60	0.198
High LTPA		114	1.0	116	0.83	0.38–1.84	0.647	31	0.46	0.10–2.11	0.315
<50 years	Low LTPA	162	1.0	176	1.04	0.62–1.76	0.880	45	1.43	0.67–3.08	0.355
<50 years	High LTPA	86	1.0	83	0.75	0.30–1.88	0.534	21	0.31	0.04–2.49	0.268
≥ 50 years	Low LTPA	108	1.0	112	1.31	0.75–2.30	0.339	27	1.45	0.60–3.47	0.407
≥ 50 years	High LTPA	28	1.0	33	1.17	0.23–5.80	0.851	10	1.22	0.11–14.3	0.872

groups according to these criteria. This represents sound hypothesis testing, but caution is still warranted when interpreting results from subgroup analyses. Although we found a significant interaction between *PPARGC1A* Gly482Ser and age in the men but not in the women, we did not observe any interaction between LTPA and the polymorphism. This is likely to be due to the lack of power to detect such an interaction in our subgroups. However, we feel that our findings are also firmly supported by other data. Replication of association studies is subject to several obstacles and confounding factors that may preclude replication. Two studies on the possible association between *PPARGC1A* Gly482Ser and type 2 diabetes and the metabolic syndrome reported negative results [11, 12]. The fact that the level of physical activity modifies the 482Ser-associated risk may explain these inconsistencies. Our investigation was performed as a population-based study in a random sample of all residents aged between 30 and 74 years in a community, giving the genetic analysis a broader generalisation compared with studies based on single families. The genotype distribution of the *PPARGC1A* Gly482Ser polymorphism found here was similar to that observed in other studies [5, 6, 10, 12]. Considering the high prevalence and possible dominant effect of 482Ser, our data may have implications for public health and prevention. A recent publication from the Study to Prevent Non-Insulin-Dependent Diabetes Mellitus (STOP-NIDDM) substantiates this view [13]. In this diabetes prevention trial, 482Ser-carriers in the placebo group had a 1.6-fold higher risk of developing type 2 diabetes, and it was only in these subjects that acarbose prevented diabetes. Although our findings are consistent with these studies, given that they are based on a subgroup, the risk of a chance finding should be considered. Further studies are warranted to confirm or disprove our results.

Although a sedentary leisure time showed a strong association with obesity in both men and women, it was only in males that 482Ser was associated with this risk. This is not an unexpected finding. There are several examples of genetic variation that exhibit such sexual dimorphism, not least the *PPARGC1A* Gly482Ser polymorphism [7]. It was recently shown that *PPARGC1A* mRNA levels are reduced in women compared with those in men [8]. It is possible that the association between *PPARGC1A* Gly482Ser and *PPARGC1A* mRNA expression, which is positively correlated with the degree of physical activity, is of greater importance in men. Since this is a cross-sectional study, such interpretation should be treated with caution until further studies, preferably prospective interventional studies, have been undertaken. Meanwhile, it is likely that the age-dependent association with obesity in the present study is explained by an age-dependent reduction in *PPARGC1A* gene expression and protein function [8]. Physical activity is known to facilitate weight loss and prevent obesity by increasing the fat oxidation rate and energy expenditure. People with a high level of LTPA often have a healthier lifestyle, including a more frequent intake of healthy food, and both a healthy diet and physical activity are known to

improve insulin sensitivity. *PPARGC1A* drives the formation of oxidative type 1 myofibres and activates genes involved in mitochondrial oxidative metabolism [14]. A reduction in type 1 myofibres and an increase in glycolytic type 2 myofibres has been associated with type 2 diabetes [15]. Differences in *PPARGC1A* mRNA expression, mitochondrial metabolism and energy expenditure are likely explanations for the protective effect of 482Gly [1].

In conclusion, our findings highlight the importance of accounting for physical activity, particularly LTPA, when studying the epidemiology of obesity. We found that the *PPARGC1A* Gly482Ser polymorphism was associated with an increased risk of obesity in physically inactive elderly males. Our results therefore support the emphasis on physical activity in the prevention of obesity.

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