Association between Trp48Arg polymorphism of the CD11c gene and risk for obesity among Iranian population

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Abstract

Background Despite assessing the expression of CD11c gene in macrophages in adipose tissues and suggesting association between the gene expressions and predisposing to obesity, the relationship of the changes in CD11c gene and its variants with obesity has not been exclusively evaluated. The present study aimed to assess the relationship between rs2230424 gene polymorphism leading a single amino acid Arginine 48 to Tryptophan interchange in CD11c gene protein chain and obesity in a sample of Iranian population.

Methods This case-control association study was performed on 247 subjects including obese individuals and a sex- and age-matched healthy non-obese individuals. After DNA extraction, the DNA sequence containing the relevant polymorphic site was amplified by polymerase chain reaction (PCR). Determining different genotypic patterns of the SNP was carried out by restriction fragment length polymorphism (RFLP) analysis. To final draft the suspected genotypes of the SNP, DNA sequencing was performed.

Results The frequency of wild genotype (TT) of Trp48Arg polymorphism of the CD11c gene in obese and non-obese groups was 97.9% and 94.6% and the frequency of heterozygous genotype (TC) was 2.1% and 5.4%, respectively with no significant difference ($p = 0.230$). None of the participants had mutant genotypic pattern of the polymorphism. There was no association of the genotypic pattern of Trp48Arg polymorphism with different underlying risk factors as well as mean laboratory parameters.

Conclusion The presence of Trp48Arg polymorphism of the CD11c gene is not associated with increased risk for obesity among Iranian population.

Keywords Obesity · Gene · Polymorphism · Genetic · CD11c

Introduction

The overall prevalence of obesity as a major healthcare problem is mainly influenced by both obesogenic genomic and

environmental factors. In this regard, identifying sub-populations with a pre-existing genetic predisposition to excess adiposity has result in better understanding pathophysiological basis of obesity [1]. There is substantial evidence for the heritability of obesity, and research in both rare and common forms of obesity has identified genes with significant roles in its etiology [2]. Some of these genes have been also associated with obesity-related comorbidities such as diabetes mellitus, coronary heart disease, metabolic syndrome, and even cancers [3]. In total, prediction of the health risks of obesity for individual patients is not straightforward, but continuing advances in understanding of genetic factors influencing obesity risk and improved diagnostic technologies mean that the future for such prediction is looking increasingly bright.

CD11c is an integrin alpha X chain protein that can be found at high levels on most human dendritic cells, as well as on monocytes, macrophages, neutrophils, and some B cells [4]. The high frequency of CD11c gene expression has been revealed in some specific pathological conditions including

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hairy cell leukemia, Crohn's disease and ulcerative colitis, deep vein thrombosis, and lymphoma [5–7]. Recently, the role of expressing CD11c gene in predisposing obesity in both animal and human models has been also suggested [8]. It has been shown that the high expression level of CD11c mRNA in subcutaneous adipose tissue made a strong contribution to models predicting TNF α in which existing pro-inflammatory properties can contribute to the pathogenesis of insulin resistance and obesity [9]. Also in some experimental studies, it has been reported a significant increase in CD11c + monocyte in adipose tissue [8]. Subsequently, other investigators reported an accumulation of CD11c + leukocytes in the adipose tissue of obese mice [10]. CD11c + leukocytes in the adipose tissue of obese mice show pro-inflammatory characteristics of classically activated macrophages 9,10 and were demonstrated to play an important role in obesity-linked adipose tissue inflammation and the development of insulin resistance [11–13].

Despite assessing the expression of CD11c gene in macrophages in adipose tissues and suggesting association between the gene expressions and predisposing to obesity, the relationship of the changes in CD11c gene and its variants with obesity has not been exclusively evaluated. Because change of hydrophobic and aromatic Tryptophan-48 residue to Arginine residue can occur on main domain of the CD11c protein near to the N-glycosylation site, it is now expected that this gene variant involved in this translocation might have an important role in functional state of this protein especially in obesity basis. Hence, the present study aimed to assess the relationship between rs2230424 gene polymorphism leading a single Arginine 48 to tryptophan interchange in CD11c gene protein chain and obesity in a sample of Iranian population.

Materials / patient and methods

Study population This case-control association study was performed on 145 obese individuals (with body mass index higher than 30 kg/m²) who referred to obesity clinic at Obesity and Eating Habits Research Center, Endocrinology and Metabolism Institute of Tehran University of Medical Sciences in 2014. A sex- and age-matched healthy group including 102 non-obese individuals were also included as the control. The control group was selected from the aesthetics clinic. The exclusion criteria were history of diabetes or other metabolic diseases as secondary causes of obesity, and history of using glucocorticoids for more than 3 months. Baseline characteristics including demographic information, medical history, anthropometric indices, and laboratory parameters were collected from the recorded files at the clinic. The objectives of the study were explained to the participants in details and verbal consent was obtained from all.

Study protocol and measurements The single nucleotide polymorphism (SNP) evaluated in this study included rs2230424 located at position 48 (W48R, p.Trp48Arg) of the CD11c gene of the chromosome chr16:31367318. Genomic DNA was isolated from white blood cells by a standard salt precipitation method. The DNA sequence containing the relevant polymorphic site was amplified by polymerase chain reaction (PCR) with a reaction mixture containing specific designed primers, Taq DNA Polymerase 2x Master, and DNA sample by Bio-Rad MJ Mini thermal cycler. Determining different genotypic patterns of the SNP was carried out by restriction fragment length polymorphism (RFLP) analysis carried out by employing primers digested by restriction enzyme *Van91I* (ER0711; Thermo Scientific). To final draft the suspected genotypes of the SNP, DNA sequencing was performed. Serum concentrations of glucose, triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), were measured by a colorimetric method using Pars Azmoon kits (Pars azmoon.co, Tehran, Iran) with an auto-analyzer (Autoanalyzer Hitachi 902, Roche Diagnostics, Holliston, MA, USA). Serum insulin concentration was measured by an enzyme-linked immunosorbent assay kit (Monobind kit, Monobind Inc., Lake Forest, CA, USA). Plasma concentration of hemoglobin HbA1c was measured using automatic analyzer DS5 and DS5 Pink Reagent kits).

Statistical analysis For statistical analysis, results were presented as mean $\pm$ standard deviation (SD) for quantitative variables and were summarized by frequency (percentage) for categorical variables. Continuous variables were compared using T test or Mann-Whitney U test whenever the data did not appear to have normal distribution or when the assumption of equal variances was violated across the study groups. Categorical variables were, on the other hand, compared using chi-square test. *P* values of ≤ 0.05 were considered statistically significant. For the statistical analysis, the statistical software SPSS version 20.0 for windows (IBM, Armonk, New York) was used.

Results

Baseline information In total, 247 patients were assessed that among them, 145 were obese and 102 were healthy non-obese individuals. Among obese ones, the mean age was 40.33 ± 12.52 years that 18.6% were male. The mean weight was 98.66 ± 22.90 kg and the mean height was 161.90 ± 8.20 cm. Regarding general risk factors, 6.9% had the history of ischemic heart diseases, 16.6% were hypertensive, 14.5% had dyslipidemia, 2.1% had history of hyperthyroidism, and 5.5% had history of hypothyroidism. Although diabetics were excluded from our study, 6.9% of patients were newly diagnosed as diabetics. Also, 15.9% had PCOS, 6.9% had gastrointestinal

Fig. 1 The segment size of Trp48Arg locus derived from the PCR

problems, and 9.0% had psychological problems. With respect to oral medications, 11% used calcium supplements, 9.7% had history of using vitamin D supplements, and 3.4% used folic acid supplements.

Genetic information The segment size of Trp48Arg locus derived from the PCR and digestion in DNA samples was all 252 bp (Figs. 1, 2 and 3). With the digestion by restrictive endonuclease *Van9II*, when restriction enzyme cutting site was at base T, fragment of 252 bp product following PCR was digested into two fragments, whose segment size was 134 bp and 118 bp. When restriction enzyme cutting site was at base C, the fragment of product following PCR could not be digested, with the original segment size 252 bp. In overall, the frequency of wild genotype (TT) of Trp48Arg polymorphism of the CD11c gene in obese and non-obese groups was 97.9% and 94.6% and the frequency of heterozygous genotype (TC)

was 2.1% and 5.4%, respectively with no significant difference ($p = 0.230$, OR; 1.16, 95%CI: 0.22–5.54). The frequency of heterozygous genotype was similar to the data presented in Iranian whole genome sequencing data available at <http://www.iranome.ir>. None of the participants had mutant genotyping pattern of the polymorphism. There was no association between the genotyping pattern of Trp48Arg polymorphism and different underlying risk factors including male gender, history of cardiac disease, diabetes mellitus, hypertension, hyperlipidemia, thyroid diseases, history of infertility, history of PCOS, and psychological problems. Table 1 shows the mean anthropometric and laboratory markers in obese patients with wild and heterogeneous patterns. In this Table also, no difference was revealed in mean age, body mass index, as well as mean levels of FBS and lipid profile between the obese individuals with wild genotype and those with heterozygous genotype.

Fig. 2 The segments size of Trp48Arg locus digestion by restrictive endonuclease *Van9II* in RFLP technique. Only one allele C (252 bp) and one kind of mutant genotype (Fig. 2) was checked out in the obese samples

Fig. 3 Frequency of genotype patterns in obese and non-obese patients

Discussion

The accumulation of macrophages in adipose tissue is a characteristic of chronic inflammation, which is often seen in obese individuals. Macrophages secrete numerous inflammatory cytokines such as TNF- α and IL-6 and monocyte chemoattractant protein-1 resulting in insulin resistance in peripheral tissues. Recent evidence has shown that dendritic cells are the source of increasing expression of CD11c in adipose tissue compared to macrophages [14–16].

Findings of our article indicate that the presence of Trp48Arg polymorphism of the CD11c gene is not associated with increased risk for obesity among Iranian population. Although the association between this polymorphism and other metabolic abnormalities such as insulin resistance has been previously demonstrated, but the relation between the polymorphism and increased risk for obesity has not been previously assessed in human models and thus to the best of our knowledge, it is the first study in this field. Other studies have shown that the expression of CD11c in adipose tissue is positively correlated with the degree of obesity, insulin resistance, and metabolic syndrome in humans [17].

However, our study could not show the increased risk for obesity in individuals with the pointed polymorphism. In the present study, the frequency of heterozygous genotype of the

Trp48Arg polymorphism was 97.9% in obese patients and 94.6% in healthy individuals with no difference. In fact, the presence of Trp48Arg polymorphism might not be a predicting indicator for increased risk for obesity in Iranian population. In our observation, the lack of mutant genotype and also the absence of the Hardy-Weinberg equilibrium was an unusual finding. On the other hand, despite high frequency of heterozygous genotype, the mutant genotype was not found in study population. In addition, the absence of mutant genotype can be due to employing a small sample size, as the main limitation of the study. In total, it seems that the presence of Trp48Arg polymorphism may not be considered as a major genetic predisposing factor to susceptible Iranian people to general obesity.

Another important finding in our survey is the lack of association between the Trp48Arg polymorphism and other underlying risk profile including gender, advanced age, and general cardiovascular and metabolic risk factors. In some previous studies, some evidences have been obtained in association between Trp48Arg polymorphism and some metabolic pathways. Tao and colleagues [9] could show increased incidence of Trp48Arg polymorphism in parallel to increase of serum level of TNF-alpha and also increase the level of HOMA-IR in PCOS women indicating a probable role of this polymorphism in triggering insulin resistance. In another study by Wu et al. [10], the presence of obesity in animal models was accompanied with the

Table 1 Mean anthropometric and laboratory markers in obese patients with wild and heterogeneous patterns

Parameter	Wild genotype	Heterogeneous genotype	<i>p</i> value
Weight (kg)	94.80	101.30	0.686
Body mass index (kg/m ²)	35.48	36.66	0.529
Fasting blood sugar (mg/dl)	100.00	101.00	0.999
Total cholesterol (mg/dl)	199.00	185.94	0.730
HDL-C (mg/dl)	42.00	44.73	0.845
LDL-C (mg/dl)	141.50	120.57	0.426
Triglyceride (mg/dl)	80.50	140.31	0.259

increase of CD11c in adipose tissue and thus by restricting diet and its-related weight loss led to lower level of monocyte CD11C. Patsouris [18] et al. indicated that the deletion of positive CD11c cells could result in rapid compensating sensitivity to insulin. Also, ablation of CD11c cells led to considerably decreasing inflammatory markers. Thus, it seems that the deletion of positive CD11c cells can be considered as a new option to treat insulin resistance in obese patients.

The number of patients included in this study was more than what was calculated.

In conclusion, the presence of Trp48Arg polymorphism is not a potential risk factor for obesity among Iranian population. Because of employing a small sample size and impossibility of assessing the relationship between the polymorphism and obesity with the presence of confounders, performing further studies with larger sample sizes are potentially recommended.

References

- Rankinen T, Sarzynski MA, Ghosh S, Bouchard C. Are there genetic paths common to obesity, cardiovascular disease outcomes, and cardiovascular risk factors? *Circ Res*. 2015;116(5):909–22.
- Willyard C. Heritability: the family roots of obesity. *Nature*. 2014;508(7496):S58–60.
- Walley AJ, Blakemore AI, Froguel P. Genetics of obesity and the prediction of risk for health. *Hum Mol Genet*. 2006;15 Spec No 2: R124–30.
- Stewart M, Thiel M, Hogg N. Leukocyte integrins. *Curr Opin Cell Biol*. 1996;7(5):690–6.
- Shelley CS, Da Silva N, Georgakis A, et al. Mapping of the human CD11c (ITGAX) and CD11d (ITGAD) genes demonstrates that they are arranged in tandem separated by no more than 11.5 kb. *Genomics*. 1998;49(2):334–6.
- Nicolaou F, Teodoridis JM, Park H, Georgakis A, Farokhzad OC, Böttinger EP, et al. CD11c gene expression in hairy cell leukemia is dependent upon activation of the proto-oncogenes ras and junD. *Blood*. 2003 May 15;101(10):4033–41.
- Bernstein CN, Sargent M, Gallatin WM. Beta2 integrin/ICAM expression in Crohn's disease. *Clin Immunol Immunopathol*. 1998;86(2):147–60.
- Caimi G, Tozzi Ciancarelli MG, Ferrara F, Montana M, Calandrino V, Canino B, et al. Deep venous thrombosis: behaviour of the polymorphonuclear leukocyte integrin pattern at baseline and after in vitro activation. *Clin Hemorheol Microcirc*. 2005;33(1):11–7.
- Tao T, Li S, Zhao A, Zhang Y, Liu W. Expression of the CD11c gene in subcutaneous adipose tissue is associated with cytokine level and insulin resistance in women with polycystic ovary syndrome. *Eur J Endocrinol*. 2012;167(5):705–13. <https://doi.org/10.1530/EJE-12-0340>.
- Wu H, Perrard XD, Wang Q, Perrard JL, Polsani VR, Jones PH, et al. CD11c expression in adipose tissue and blood and its role in diet-induced obesity. *Arterioscler Thromb Vasc Biol*. 2010 Feb;30(2):186–92.
- Brake DK, Smith EO, Mersmann H, Smith CW, Robker RL. ICAM-1 expression in adipose tissue: effects of diet-induced obesity in mice. *Am J Phys Cell Physiol*. 2006;291:C1232–9.
- Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest*. 2007;117:175–84.
- Nguyen MT, Favellyukis S, Nguyen AK, Reichart D, Scott PA, Jenn A, et al. A subpopulation of macrophages infiltrates hypertrophic adipose tissue and is activated by free fatty acids via toll-like receptors 2 and 4 and JNK-dependent pathways. *J Biol Chem*. 2007;282: 35279–92.
- Olefsky JM, Glass CK. Macrophages, inflammation, and insulin resistance. *Annu Rev Physiol*. 2010;72:219–46.
- Lumeng CN, Saltiel AR. Inflammatory links between obesity and metabolic disease. *J Clin Invest*. 2011;121:2111–7.
- Stefanovic-Racic M, Yang X, Turner MS, Mantell BS, Stolz DB, Sumpter TL, et al. Dendritic cells promote macrophage infiltration and comprise a substantial proportion of obesity-associated increases in CD11c+ cells in adipose tissue and liver. *Diabetes*. 2012;61:2330–9.
- Wentworth JM, Naselli G, Brown WA, Doyle L, Phipson B, Smyth GK, et al. Pro-inflammatory CD11c+CD206+ adipose tissue macrophages are associated with insulin resistance in human obesity. *Diabetes*. 2010;59:1648–56. Also, CD11c macrophages have been shown to induce high expression of integrins in obese females, and integrins such as $\alpha 5$ may be involved in activating inflammatory signaling in adipose tissue [18].
- Patsouris D, Li PP, Thapar D, Chapman J, Olefsky JM, Neels JG. Ablation of CD11c-positive cells normalizes insulin sensitivity in obese insulin resistant animals. *Cell Metab*. 2008;8:301–9.