INTERLEUKIN-1 RECEPTOR ANTAGONIST GENE POLYMORPHISM AND OBESITY: A PILOT STUDY FROM NORTH INDIA

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ABSTRACT

The main adverse consequences of excess bodyweight are cardiovascular disease, type II diabetes, and several cancers. IL-1Ra serum concentration has been reported earlier to increase in human obesity and it is therefore assumed that the polymorphism of IL-1Ra may influence cytokine production. We designed this study to investigate whether the IL-1Ra polymorphism was associated with obesity. A total number of 103 individuals; 19 lean (BMI <25 Kg/m²), 51 overweight (BMI 25-29.9 Kg/m²) and 33 obese (BMI ≥ 30.0 Kg/m²) were enrolled in this study. Genotyping was performed using a polymerase chain reaction PCR amplification of the intron-2 fragment harboring a variable number of tandem repeat (VNTR) nucleotide sequences 86 bp of tandem repeat. The PCR products were separated on 2% agarose gel. Statistical analysis was performed using SPSS software (version 11.5). We found no significant difference in genotype and allele frequencies between the three groups; lean vs. overweight and lean vs. obese ($p=0.323$; 0.202 ; 0.123 and 0.068 resp). However, an increased risk for obesity had a propensity to be higher in those having genotype II/II. This genotype has been reported to be a 'high producer' of IL-1Ra. Although no statistically significant relationship between IL-1Ra polymorphism and BMI was observed, however, a trend towards an increase of allele *II in overweight and obese group was observed. This may suggest that IL-1Ra appears to be induced by inflammatory stimuli as well as obesity-associated factors. This is relatively a pilot study; but nevertheless, may assist in identifying the pathophysiological cause for obesity.

KEY WORDS

IL-1 Ra Polymorphism, VNTR, Single nucleotide polymorphisms (SNP), Obesity

INTRODUCTION

Obesity is an important cause of morbidity and mortality worldwide. It is defined as a body mass index (BMI) of greater than 30 kg/m² and is associated with many diseases such as type 2 diabetes mellitus, hypertension, coronary heart disease, dyslipidemia, gall bladder diseases, infertility and certain forms of cancer (1). As a complex disease, it is determined by multiple genetic and environmental factors, including physiological, behavioral, and socio-cultural

factors (2,3).

Cytokines appear to be major regulators of adipose tissue metabolism (4). Expression studies show that adipocytes can synthesize tumor necrosis factor alpha (TNF- α) and several interleukin (IL) notably IL-1 β and IL-6. IL-1 β is well known to suppress adipocyte differentiation and lipoprotein lipase expression and activity (5) by inhibiting the expression of fatty acid transport protein in adipose tissue (6). In addition, IL- β is significantly more potent relative to TNF- α and other cytokines in inducing anorexia (7). IL-1Ra is markedly up regulated in the serum of obese patients, is correlated with BMI and insulin resistance (8). It is an important regulator of adipogenesis, food intake, and energy expenditure (9)

IL-1 consists of IL-1 β and IL-1Ra. Genes for IL-1 β and IL-1Ra are located on 2q14-21. Five alleles of the IL-1Ra gene have been reported, corresponding to 2,3,4,5 and 6 copies of an

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86-base pair sequence repeats located in intron-2 (10).

IL-1Ra is an endogenous inhibitor that antagonizes many of the biological actions of IL-1 β by competitive inhibition (11). In addition, IL-1Ra serum concentrations are highly increased in human obesity and that its concentrations decrease after weight loss (12). Therefore, it is likely that polymorphism of the IL-1Ra be affected due to variation in cytokine production. It is for this reason we hypothesized IL-1Ra gene as a candidate gene for obesity to investigate whether this polymorphism is associated with obesity in a cohort of north Indian population.

MATERIALS AND METHODS

Subjects : A total number of 103 subjects (age range, 19-67) recruited from King George Medical University, Lucknow, were included in the present study. The patients in the study had no history of metabolic disorder, renal, liver, hematological disease or any other disorder other than obesity. The subjects were divided into three body mass index (BMI) groups according to World Health Organisation (WHO) definitions: lean (BMI <25 Kg/m²), overweight (BMI 25-29.9 Kg/m²) and obese (BMI $\geq$ 30.0 Kg/m²). In the present study BMI ranged between 19 and 50.7 Kg/m². Informed consent was obtained from the patients and the controls participating in the study. The hospital ethical committee approved the study.

Phenotype measurements : BMI was calculated as weight (Kg)/height (cm) square. Waist circumference (measured at narrowest point to the hip) was divided by the circumference of the hip (measured at its greatest gluteal protuberance) to

obtain the waist- to-hip ratio (WHR).

DNA extraction : Five ml of blood was collected in EDTA vials from cases and controls. DNA was extracted from blood lymphocytes using 'salting out' method (13).

IL-1Ra VNTR genotyping : The primer sequences used were: forward, 5'- CTCAGCAACACTCCTAT-3'; reverse, 5'- TCCTGGTCTGCAGGTAA-3'. The region within the second intron of IL-1Ra gene contains variable number of tandem repeat (VNTR) of 86 base pairs. The PCR products of 410 bp (allele I= four repeats), 240 bp (allele II= two repeats), 500 bp (allele III= five repeats), 325 bp (allele IV= three repeats), 595 bp (allele V= six repeats) and 154 bp (allele VI= one repeat) were analyzed by electrophoresis on a 2% agarose gel (14).

PCR conditions : Initial denaturation, 95°C for 5 min followed by 95°C for 30 sec, annealing at 58°C for 30 sec, extension at 72°C for 30 sec for 30 cycles and a final extension at 72°C for 10 min. The size of PCR product was determined using a 100-bp DNA ladder (Roche, Germany). The molecular weight of each band was determined by using software in Alpha Imager 1220 version 5.5 programme.

Statistical Analysis : Statistical analysis was performed using the χ^2 test to compare the genotype and allelic frequency distribution in lean, overweight and obese with the SPSS software (version 11.5). Allele and genotype frequencies were compared using a 2x2 contingency table using Fisher's exact test. The mean concentration of all numerical values was tested by the Student *t*-test or ANOVA test. *P*- value <0.05

Table 1 : Characteristics of subjects according to BMI

	BMI (Kg/m ²)		<i>p</i> ^a	
	Lean (<25)	Overweight (25-29.9)	Obese ($\geq$ 30.0)	
N (%)=103	19	51	33	
Age (years)	37.3 $\pm$ 9.3	39.2 $\pm$ 12.2	36.5 $\pm$ 12.0	
Gender: male	10	23	16	NS
Female	9	28	17	
Weight (Kg)	65.7 $\pm$ 8.9	75.4 $\pm$ 7.9	91.7 $\pm$ 16.6	0.000
Height (cm)	166.3 $\pm$ 8.7	164.7 $\pm$ 8.9	163.8 $\pm$ 9.3	NS
BMI	23 $\pm$ 1.8	27.5 $\pm$ 1.25	33.6 $\pm$ 3.9	0.000
WHR	0.86 $\pm$ 0.11	0.89 $\pm$ 0.02	0.90 $\pm$ 0.11	NS
SBP (mm Hg)	108.6 $\pm$ 10.15	117.6 $\pm$ 9.8	123.6 $\pm$ 14.1	0.001
DBP (mm Hg)	71.0 $\pm$ 8.1	77.7 $\pm$ 7.9	80.8 $\pm$ 9.1	0.002

Mean $\pm$ SD; BMI: body mass index; WHR: waist-to-hip ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure; NS: non-significant.

^a one way ANOVA test

Table 2 : Distribution of IL-1Ra gene polymorphism in lean, overweight and obese group

	Lean (n=19)		Overweight (n=51)		Obese (n=33)	
	n	(%)	n	(%)	n	(%)
<i>Genotype frequencies</i>						
I/I 410	7	(36.8)	9	(17.6)	5	(15.2)
II/II 240	10	(52.6)	33	(64.7)	23	(69.6)
I/II 410/240	1	(5.3)	7	(13.7)	5	(15.2)
I/III 410/500	1	(5.3)	2	(4)	0	(0)
<i>Allelic frequencies</i>						
Allele I	15	(39.5)	25	(24.5)	15	(22.7)
Allele II	22	(57.9)	75	(73.5)	51	(77.3)
Allele III	1	(2.6)	2	(2)	0	(0)

Genotype frequencies : Lean vs. overweight: $p=0.323$; $\chi^2=3.485$; $df=3$
 Allelic frequencies: Lean vs. overweight: $p=0.202$; $\chi^2=3.205$; $df=2$

Lean vs. obese: $p=0.123$; $\chi^2=5.770$; $df=3$
 Lean vs. obese: $p=0.068$; $\chi^2=5.371$; $df=2$

were considered statistically significant. Odds ratio (OR) at 95% confidence intervals (CI) was determined for the disease susceptibility of patients.

RESULTS

The clinical characteristics of all the subjects are given in Table 1. The frequency distribution of different genotypes and allelic frequencies in lean, overweight and obese are shown in Table 2. The genotype distribution of IL-1Ra polymorphism was in Hardy-Weinberg equilibrium. The bands on 2% agarose gel revealed variable number of tandem repeats (Figure 1). The fragment sizes for IL-1Ra do not correspond with the number of repeats as some extra bases are also amplified along with the repeats. In the present study, we have not found allele IV (325 bp), allele V (595 bp) and allele VI (154 bp) in any of the

three groups. The genotype and allelic frequencies did not differ significantly between lean vs. overweight and lean vs. obese (p-values: 0.323; 0.202; 0.123 and 0.068 respectively). Although, the presence of genotype 240/240 showed > 2 folds risk in overweight and >3 folds in obese (OR=2.722; 95% CI=0.838-8.840 & OR=3.267; 95% CI=0.862-12.377 respectively).

DISCUSSION

Obesity is a major chronic disorder affecting 20-40% adults in India. It is a complex metabolic disorder that is genetically determined. There are many candidate genes for obesity that affect the adipocyte differentiation (15). The present study was undertaken to determine whether VNTR polymorphism of IL-1Ra gene was associated with obesity in north Indian population. We compared the genotype frequencies of lean with that of overweight and obese groups and found no significant association. Though the p-value was non-significant between lean and obese, Odds ratio when calculated, higher risk > 2 folds in overweight and >3 folds in obese ('high producers' of IL-1Ra) was observed. Our results are in accordance to Um *et al.* (4) who also found no relationship between IL-1Ra polymorphism and BMI in Korean population. Proinflammatory cytokines like IL-1 β and TNF- α suppresses adipose differentiation and lipoprotein lipase expression (6). Bruun *et al.* demonstrated that TNF- α and IL-1 β are able to regulate the production and release of leptin from human adipose tissue fragments in vitro (16). In addition, Plata-Salaman *et al.* (7) reported that obese rats show a significantly stronger anorexia in response to the central administration of IL-1 β than do lean controls. These findings support our

Fig. 1. IL-Ra: Lane M: Molecular marker (100 bp), Lane 1: 410/410 bp (I/I), Lane 2: 240/240 bp (II/II), Lane 3: 410/240 bp (I/II), Lane 4: 410/500 bp (I/III)

hypothesis that the increased basal expression of IL-1 β as a consequence of the polymorphism can be protective against being overweight but the presence of genotype II/II of IL-1Ra that is a 'high producer' therefore counteracts the properties of IL-1 β and may thus increase the adipocyte differentiation and promote lipogenesis in adipose tissue (17). IL-1Ra also plays a regulatory role in energy homeostasis. Luheshi *et al.* (18) demonstrated that the hypothalamic effects of leptin depend heavily on the action of IL-1 and that the injection of IL-1Ra into the cerebral ventricles inhibited the leptin-induced reduction in food intake as well as the concomitant increase in body temperature by more than 60% (8). As the central resistance to leptin, rather than its deficiency, is the hallmark of most cases of human obesity, a better understanding of the factors involved in the regulation of the hypothalamic sensitivity to leptin is important. Thus, based on these findings, we focused on the association between the polymorphism of IL-1Ra and obesity. However, more knowledge about genetics of obesity is needed in order to prevent it in existing and next generation.

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REFERENCES

- Kopelman PG. Obesity as a medical problem. *Nature* 2000; 404: 635-43.
- Liu YJ, Araujo S, Recker RR, Deng HW. Molecular and genetic mechanisms of obesity: implications for future management. *Curr Mol Med* 2003; 3: 325-40.
- Um JY, Kim HM, Mun SW, Song YS, Hong SH. Interleukin-1 receptor antagonist gene polymorphism and traditional classification in obese women. *Int J Neurosci* 2006; 116: 39-53.
- Um JY, Chung HS, Song MY, Shin HD, Kim HM. Association of interleukin-1beta gene polymorphism with body mass index in women. *Clin Chem* 2004; 50: 647-50.
- Price SR, Mizel SB, Pekala PH. Regulation of lipoprotein lipase synthesis and 3T3-L1 adipocyte metabolism by recombinant interleukin 1. *Biochim Biophys Acta* 1986; 889: 374-81.
- Memon RA, Feingold KR, Moser AH, Fuller J, Grunfeld C. Regulation of fatty acid transport protein and fatty acid translocase mRNA concentrations by endotoxin and cytokines. *Am J Physiol* 1998; 274: 210-17.
- Plata-Salaman CR, Vasselli JR, Sonti G. Differential responsiveness of obese (fa/fa) and lean (Fa/Fa) Zucker rats to cytokine-induced anorexia. *Obes Res* 1997; 5: 36-42.
- Somm E, Cettour-Rose P, Asensio C, Charollais A, Klein M, Theander-Carrillo C, et al. Interleukin-1 receptor antagonist is upregulated during diet-induced obesity and regulates insulin sensitivity in rodents. *Diabetologia* 2006; 49: 387-93.
- Somm E, Henrichot E, Pernin A, Juge-Aubry CE, Muzzin P, Dayer JM, et al. Decreased fat mass in interleukin-1 receptor antagonist-deficient mice: impact on adipogenesis, food intake, and energy expenditure. *Diabetes* 2005; 54: 3503-9.
- Tarlow JK, Blakemore AIF, Lennard A, Solari R, Hughes HN, Steinkasserer A, et al. Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86 base pair tandem repeat. *Human Genet* 1993; 91: 403-4.
- Dinarello CA. The interleukin-1 family: 10 years of discovery. *FASEB J* 1994; 8: 1314-25.
- Meier CA, Bobbioni E, Gabay C, Assimacopoulos-Jeannet F, Golay A, Dayer JM. IL-1 receptor antagonist serum concentrations are increased in human obesity: a possible link to the resistance to leptin? *J Clin Endocrinol Metab* 2002; 87: 1184-8.
- Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1998; 16: 1215.
- Bioque G, Crusius JBA, Koutroubakis I, Bouma G, Kostense PJ, Meuwissen SGM, Pena AS. Allelic polymorphism in IL-1 β and IL-1receptor antagonist (IL-1Ra) genes in inflammatory bowel disease, *Clin Exp Immunol* 1995; 102: 379-83.
- Arner P. Obesity a genetic disease of adipose tissue? *Br J Nutr* 2000; 83: S9-S16.
- Bruun JM, Pedersen SB, Kristensen K, Richelsen B. Effects of pro-inflammatory cytokines and chemokines on leptin production in human adipose tissue in vitro. *Mol Cell Endocrinol* 2002; 190: 91-9.
- Hurme M, Santtila S. IL-1 receptor antagonist (IL-1RA) plasma levels are co-ordinately regulated by both IL-1R and IL-1beta genes. *European Journal of Immunology*; 28: 2598-2602.
- Luheshi GN, Gardner JD, Rushforth DA, Loudon AS, Rothwell NJ. Leptin actions on food intake and body temperature are mediated by IL-1. *Proc Natl Acad Sci USA* 1999; 96: 7047-52.