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# **The Association of Adiponectin Gene Polymorphisms (-11391G/A), (+45T/G) With WC and Type 2 Diabetes in North East India**

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**Abstract: Background:** Adiponectin is a protein, secreted from adipose tissue, according to numerous studies that have been done before, its plasma levels are associated with different parameters of metabolic syndrome. It seems that this effect results from the interaction of genetic (polymorphisms in the adiponectin gene) and environmental factors (obesity). The present study aimed to investigate the possible association between two SNPs of adiponectin gene at position (+45T/G) and (-11391G/A) with increased waist circumference (WC) and type 2 diabetes mellitus patients in North East, Guwahati, India.

**Method:** This study is a case - control study with 121 diabetic patients were selected as cases group and 87 healthy people selected as controls group. After extraction of DNA, genotyping was performed by PCR-RFLP method.

**Results:** We don't observed a significant association between adiponectin gene polymorphism (+45T/G) and waist circumference (WC). In diabetic patients over 40 years old we observed a significant association between adiponectin gene polymorphism (-11391 G/A) and waist circumference(WC) ( $P = 0.021$ ).

**Conclusion:** It seems that the role of adiponectin polymorphism (+45 T / G) in the creation of type 2 diabetes independent to abdominal obesity and adiponectin polymorphisms of (-11391G / A) in type 2 diabetes depend to abdominal obesity in people older than 40 years (waist circumference <90).

**Keywords:** Adiponectin, Polymorphisms, Type2 diabetes, WC, Abdominal obesity.

## **I. INTRODUCTION**

Today, obesity Related to nutrition and creation of metabolic syndrome has emerged as a global health problem[1,2]. Adiponectin which is exclusively secreted from adipose tissue and its plasma level decrease inversely with increased fat mass[3,4], the metabolic effects is to glucose and lipid metabolism [5], anti-inflammatory effects[3] and anti-atherogenic[6] and these effects exerts by sensitizing the body to insulin[7,8]. In several studies, plasma levels of adiponectin has decreased in the obese animal models[9,10] and human models especially viscera[11,12,13]. The decrease of plasma level of adiponectin, is dependent on several factors such as production reduction or increased clearance from the blood by the liver. In addition, the genetic factors effecting on plasma levels of adiponectin[ such as SNP adiponectin gene], and environmental factors such as removing high-fat diet which are delays for blood adiponectin cleaning[14], and carbohydrate-rich diet is related with lower levels of adiponectin[15] also seems to have influence on adiponectin levels. However, little studied have done about the effect of diet on adiponectin levels but studies shows that it has a better effect on the long-term regimes[16,17]. In some studies, the relation between SNP adiponectin gene and plasma level of adiponectin approved[18,19,20]. It is hypothesized that the diseases such as type 2 diabetes, which is caused the cumulative effect of genetic disorders[SNPs adiponectin gene] with environmental factors [obesity] [21]. Association of adiponectin polymorphisms in skinny people with type 2 diabetes is statistically meaningless [22].

However, the association of adiponectin gene polymorphisms with type 2 diabetes and obesity is vary in different populations. There are many SNPs of adiponectin have been extensively studied which are including: -11391G/A, -11377G/C, +45T/G and 276G/T [26]. The purpose of this study is to investigate the relation between adiponectin gene polymorphisms (+45T/G and -11391G/A) with waist circumference (WC) and type 2 diabetes in the India population .

## **II. MATERIALS AND METHODS**

### **A. Sample Collection**

In this study, 5 ml blood samples were collected in EDTA vials from 121 northeast women patient with type 2 diabetic diagnosed at the Northeast Cancer Hospital, Assam, India and 87 healthy Northeast women from Health Screening Center of Northeast Cancer

## International Journal for Research in Applied Science & Engineering Technology (IJRASET)

Hospital, Assam, India between the period February 2013 to December 2014, Information regarding sex, current age, age at type 2 diabetes diagnosis, and ethnic status was recorded. The study has been approved by Gauhati University ethical committee, and the written informed consent to participate [i.e., case and controls] in the study was obtained from all subjects.

### B. DNA isolation and PCR-RFLP

Salting out extraction method was used to isolate genomic DNA from the collected blood, and the quantity of DNA was determined by spectrophotometer. Amplification of the gene polymorphism regions was carried out by Polymerase Chain Reaction (PCR), and the Restriction Fragment Length Polymorphism (RFLP) was used to identify the Single nucleotide polymorphism regions (SNPs) of Adiponectin (45T/G and -11391G/A). The PCR primer sequences (Invitrogen, India) used for Adiponectin SNPs (45T/G and -11391G/A) are shown in Table 2. PCR was standardized and carried out for about 34 cycles. The PCR products were separated on 1.5% agarose gel, visualized with ethidium bromide. The different restriction enzymes (genome diagnostics private limited, India) used to study the respective gene polymorphisms by RFLP method was mentioned in Table 3. The restriction enzyme digestion was carried out for 37C (overnight; 16 hours), and the products were visualized on 2% agarose gel stained with ethidium bromide (Figure 1 and 2).

### C. Statistical analysis

The allele frequency differences between case and control groups were obtained using used  $\chi^2$  (Chi-square) test. For each SNP the odds ratios (ORs) with confidence intervals (CIs 95%) were calculated, and the identification of genotypes risk was performed using logistic regression analysis. Pearson  $\chi^2$  statistics with threshold of  $P < 0.05$  for each SNP was used to test the Hardy-Weinberg equilibrium. SPSS 16 software was used to perform the statistical analysis and the possibility less than 0.05, was considered significant. Power and Sample Size Calculations was used to estimate the Power (<http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize>).

## III. RESULT

In this study, the age of patients group are  $55 \pm 9$  years and for controls group are  $50 \pm 5$  years. Standard values for waist circumference (WC) are  $92 \pm 8$  cm for patients group and  $96 \pm 10$  cm for controls group. In both diabetic and non-diabetic, the difference frequency in genotype various of +45T/G position adiponectin gene compared to those with higher than 90 cm WC and lower than 90 cm WC was not statistically significant (Table1). In position -11391G/A of adiponectin gene the result was same also (Table1).

This study were conducted the people over 40 years and the results were invalid in positions +45T/G, but there was a statistically significant difference in position -11391G/A (Table2). This means that in people over 40 years, the GG genotype frequencies of -11391G/A polymorphism in patients with over that 90cm WC was significantly higher than people who have WC below 90cm (CI 95% = 1/01 – 16, OR= 3/61, P= 0/02).

Comparison of GG genotype frequencies of -11391 polymorphisms in diabetes patients according to WC index showed significant differences.

## IV. DISCUSSION

The purpose of this study was to examine two common polymorphisms -11391G/A and +45T/G of adiponectin with WC in diabetic and non-diabetic people. Waist circumference (WC) was considered as an obesity index. According to ATP III, the WC more than 102cm and, in WHO, the WC more than 37 inch have abdominal obesity [27,28]. In this study the WC more than 90cm was considered as abdominal obesity. In other study, allele A in -11391G/A polymorphism only in women and +45T/G polymorphism in both gender women and men has protective role against obesity. The effect of -11391A polymorphism is conformed in French population, In a study in Denmark have seen associated between -11391A polymorphism and obesity [24]. In Hispanic population the study of 18 SNPs of adiponectin were evaluated with different criteria for obesity such as , BMI, WC, Ratio of WC to hip, Subcutaneous adipose tissue, Visceral adipose tissue, The ratio of visceral fat to subcutaneous fat which have seen relation between 7 polymorphisms with obesity[29]. In a non-diabetic Korean population have seen relation between +45T/G polymorphism and serum level of adiponectin, insulin resistance and obesity[23]. Similar to Korean population study, it was reported in Italy population study also between +276 and +45 polymorphisms with insulin resistance and other related index[ 25].

In this study we observed that the type 2 diabetes is more happened in obese people over 40 years old with 11391G/A polymorphism of adiponectin in Indian population, which is similar to French study, Denmark study and Hispanic study [20,24,29].

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we recommended similar studies with larger population in other polymorphisms of adiponectin on BMI, WC and it's association with type 2 diabetes.

### V. CONCLUSION

In this study we investigated the association of adiponectin gene SNPs (45T/G and -11391G/A) and their possible association with WC and Type 2 Diabetes in a Northeast women population. Our results indicates that the role of adiponectin polymorphism (+45 T/G) in the creation of type 2 diabetes independent to abdominal obesity and adiponectin polymorphisms of ( -11391G / A) in type 2 diabetes depend to abdominal obesity in people older than 40 years (waist circumference <90). To our knowledge, our study provides information on the adiponectin SNPs (45T/G and -11391G/A) and the risk WC and type 2 diabetes in Northeast women population.

### VI. ACKNOWLEDGEMENTS

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Table 1: Polymorphism genotype frequencies -11391G/A and +45T/G of adiponectin gene in non-diabetic and diabetic patients groups by WC index

Gene	Genotype	Diabetic patients		Non-Diabetic patients	
		WC>90	WC≤90	WC>90	WC≤90
+45	TT	86 [71/4]	84[69/8]	64 [73/1]	52 [60]
	TG	33 [27]	32 [26/4]	20 [23/1]	26 [30]
	GG	2[1/6]	5 [3/8]	3[3/8]	10 [10]
-11391	GG	115 [95/3]	110 [90/6]	79 [90/9]	82 [94/1]
	GA	4 [3/1]	11 [9/4]	8 [9/1]	5 [5/9]
	AA	2 [1/6]	0[0]	0[0]	0[0]

No significant differences were observed.

Table 2: Polymorphism genotype frequencies -11391G/A and +45T/G of adiponectin gene in non-diabetic and diabetic patients groups in over 40 years old patients by WC index

Gene	Genotype	Diabetic patients		Non-Diabetic patients	
		WC>90	WC≤90	WC>90	WC≤90
+45	TT	86 [70/7]	86 [70/8]	63 [72/9]	50 [57/9]
	TG	31 [25/9]	30 [25]	22 [25]	28 [31/6]
	GG	4 [3/4]	5 [4/2]	2 [2/1]	9 [10/5]
-11391	GG	117[96/6]	108 [89/6]	79 [90/5]	82 [93/75]
	GA	4 [3/4]	13 [10/4]	8 [9/5]	5 [6/25]
	AA	0[0]	0[0]	0[0]	0[0]

In Chi-square test  $P \leq 0/05$  was statistically significant.

Table 3: PCR primer sequences and the restriction enzyme digestion used to study the gene polymorphisms

Gene	Polymorphisms	PCR primer sequence [F = Forward , R= Reverse ]	Restriction enzymes used for RFLP
Adiponectin	+45T/G	F : 5'- GAAGTAGACTCTGCTGAGATGG - 3' R : 5'- TATCAGTGTAGGAGGTCTGTGATG - 3'	SmaI
	-11391G/A	F : 5'- CATCAGAATGTGTGGCTTGC - 3' R : 5'- AGAAGCAGCCTGGAGAACTG - 3'	MspI



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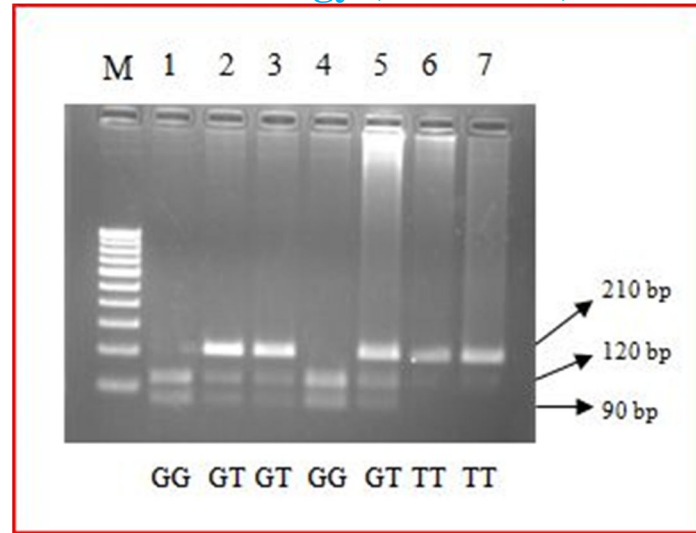


Figure 1: Identification of Adiponectin polymorphism. SNP 45G/T: SmaI restriction fragments. Lanes no. 6 & 7 were homozygous wild type [genotyped TT] [210 bp], lanes no. 2 & 3 & 5 were heterozygous mutant [genotyped TG] [210 bp, 120bp, 90 bp] and lanes no. 1 & 4 were homozygous mutant [genotyped GG] [120 bp, 90 bp]. M, 100 bp DNA ladder.

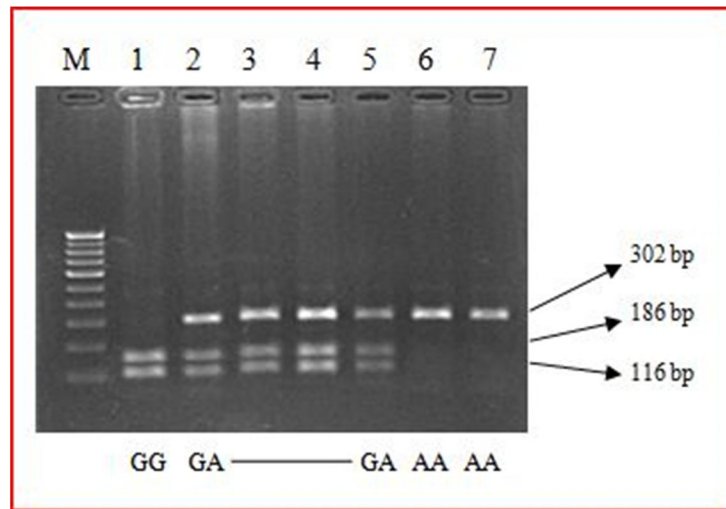


Figure 2: Identification of Adiponectin polymorphism. SNP 11391G/A: MspI restriction fragments. Lanes no. 2,3,4,5 were heterozygous mutant [genotyped GA][ 302 bp, 186bp, 116 bp], lane no. 1 was homozygous wild type [genotyped GG] [186 bp, 116 bp] and lanes no. 6,7 were homozygous mutant [genotyped AA] [302 bp]. M, 100 bp DNA ladder.

### REFERENCES

- [1] Reaven, G.M. The Insulin resistance syndrome: Definition and dietary approach to treatment. *Annu Rev Nutr* 2005; 25:391-406.
- [2] Despres J.P. Health consequences of visceral obesity. *Ann Med* 2001; 33: 534-541.
- [3] Hotamisligil G.S, Shargill N.S, Spiegelman B.M. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 1993; 259: 87-91.
- [4] Yang Q, et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature* 2005; 436: 356-362.
- [5] Kershaw E.E, Flier J.S. Adipose tissue as an endocrine organ *J Clin Endocrinol Metab* 2004; 89: 2548-2556.
- [6] Lara-Castro C, Fu Y, Chung BH, Grady WT. Adiponectin and the metabolic syndrome: mechanisms mediating risk for metabolic and cardiovascular disease. *Curr Opin Lipidol* 2007; 18: 263-270.
- [7] Yamauchi T, Kamon J, Minokoshi Y, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 2002; 8[11]: 1288-95.
- [8] Berg AH, Combs TP, DU X, et al. The adipocyte-secreted protein Acrp 30 enhances hepatic insulin action. *Nat Med* 2001; 7[8]: 947-53.
- [9] Hu E, Liang P, Spiegelman B.M. Adipo Q is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem* 1996; 271: 10697-10703.

## International Journal for Research in Applied Science & Engineering Technology (IJRASET)

- [10] Hottak,et.al.Circulating concentrations of the adipocyteprotein adiponectin Are decreased in parallel with reduced insulin sensitivity during the progression to type2Diabetes in rhesus monkeys. *Diabetes* 2001; 50: 1126-1133.
- [11] Ryo M,et al Adiponectin as a biomarker of the metabolic syndrome.*Circ J* 2004;64:975-981.
- [12] Yatagai T, et al. Hypoadiponectinemia is associated with visceral fat accumulation and insulin resistance in Japanese men with type2 diabetes mellitus. *Metabolism* 2003; 52: 1274-1278.
- [13] YamamotoY, Hirose H, SaitoL et al. Adiponectin, an adipocyte-driven Protein, predicts future insulin-resistance: two-year fallow up study in Japa nese population.*J Clin Endocrinol Metab* 2004;89: 87-90.
- [14] Nils Halberg, Todd D. Schraw, Zhao V. Wang etal. Systemicfate of the adipocyte-derived factor adiponectin. *Diabetes* 2009; 58[9]: 1961-1970
- [15] Hye Kyung Chung, RD, Jey Sook Chae et al. influence of adiponectin gene polymorphisms on adiponectin level and insulin resistance index in response to dietary intervention in overweight-obese patients with impaired fasting glucose or newly diagnosed type2 diabetes *Diabetes care* 2009; 32[4]:552-558.
- [16] Pischon T, Girmon CJ, Rifai N, et al. Association concentration in men. *Am J Clin Nutr* 2005; 81: 780-786.
- [17] Nelson TL,Steven s JR,HickeyMS.Adiponectin levels are reduced, independent of polymorphisms in the adiponectin gene,after supplementation with alpha-linolenic acid among healthy adults. *Metabolism* 2007; 56[9]: 1209-15
- [18] Pollin TL,Tanner K,OconnellJRet al.Linkage of plasma adiponecitr levels to 3q27 explained by association with variation in the APM1gene. *Diabetes* 2005; 54: 268-274.
- [19] Heid IM,Wagner SA,GohlkeHet al.Genetic architecture of the APM1 gene and its influence on adiponectin plasma levels and parameters of the metabolic syndrome in 727 healthy caucasins. *Diabetes* 2006; 55: 375-384
- [20] Bouatia-Naji N,Meyer D,Lobbens S et al. ACDC/ Adiponectin polymorphisms are associated with severe childhood and adult obesity. *Diabetes* 2006; 55:545-550
- [21] Altan Onat,GulayHergenc,Dursun Dursunoglu etal. Relatively high levels of serum adiponectin in obese women, a potential indicator of anti inflammatory-dysfunction :relation to sex hormone-binding globulin. *Int J Biol Sci*2008; 4[4]:208-214.
- [22] Crimmins NA, MartinJ. Polymorphisms in adiponectin reseptor genes ADIPOR1 and ADIPOR2 and insulin resistance.*Obes Rev* 2007;8:419-23.
- [23] Jang Y,Chae JS,Koh SJ,Hyon YJ,KimJY,Jeong YJ,Park S,Ahn CM,LeeJH:The influence of the adiponectin gene on adiponectin concentrations and parameters of metabolic syndrome in nondiabetic Korean women .*Clin Chim Acta* 2008;391: 85-90.
- [24] Lanko LB, siddiq A, lecoeur C, et al. ACDC/adiponectin and PPAR-gamma gene polymorphisms:Implications for features of obesity. *Obes Res* 2005; 13[12]; 2113-21.
- [25] Claudia Menzaghi, Tonino Ercolino et al. A Haplotype at the Adiponectin locus Is associated with obesity and other features of the insulin Resistance Syndrome. *Diabetes* 2002; si:2306-2312.
- [26] Gable OR,Matin J ,WhittallR,et al.Common adiponectin gene variants show different effect on risk of cardiovascular disease and type2 diabetes in European subjects .*Ann Hum Genet* 2007;71[pt4]:453-66.
- [27] The expert committee on the diagnosis and classification of diabetes mellitus .fallow up report on the diagnosis of diabetes mellitus. *Diabetes care* 2003;26[11]:3160-7
- [28] James B Meigs. The metabolic syndrome [insulin resistance syndrome or syndrome X] 2009 Up to date [uptodate.com].
- [29] Schaffler A, Buchler C, Muller-Lader U, et al. Identification of variables influencing resistant serum levels in patients with type1 and type2 diabetes mellitus. *Horm Metab Res* 2004;36[10]: 702-7.



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