

Association of GNB3 C825T polymorphism with obesity in Saudi population

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Abstract: The prevalence of obesity is increasing in Saudi Arabia. The search for genes that increase the susceptibility to develop obesity has become important. One set of candidate genes for obesity is the heterotrimeric G proteins, which are key components of intracellular signal transduction and play a focal role in adipogenesis. The aim of this study was to study the association between the C825T (C-to-T substitution at nucleotide 825 in exon 10) (rs5443) polymorphism of the GNB3 gene and obesity in a sample Saudi population. Blood samples from 116 healthy volunteers in age group 18-60 years were taken and obesity status was determined by the Body Mass Index (BMI). DNA was extracted from whole blood and PCR for the GNB gene was done. The PCR product thus obtained was subjected to restriction analysis using the enzyme *Bsa*II to determine the presence or absence of the GNB3 C825T polymorphism. Alleles T represent the absence of restriction site while alleles C indicate the presence of restriction site. Association between the genotypes and obesity was determined. We found that the presence of the T allele was a major contributing factor to obesity because from our study group we observed a higher occurrence of TT genotype in obese and overobese people while the normal people had a high occurrence of CT genotype and the underweight people had a high occurrence of CC genotype. Genotyping studies clearly showed that the T allele was a major contributing factor towards obesity. Higher T allele frequency was associated with obesity as compared with normal individuals.

[Taha Kumosani, Archana Iyer, Soonham Yaghmoor, Magda Hagraas, Yasmeen Hettari. **Association of GNB3 C825T polymorphism with obesity in Saudi population.** *Life Sci J* 2014;11(10):282-285] (ISSN:1097-8135). <http://www.lifesciencesite.com>. 40

Keywords: Obesity; GNB gene; C825T polymorphism; RFLP

1. Introduction

There is currently a worldwide epidemic of obesity and up to 58% of the world's adult population are predicted to be overweight or obese by 2030 (Kelly et al., 2008). Saudi Arabia is a Middle Eastern country which has gone through significant economical development over the last 20 years. The prevalence of obesity is increasing in Saudi Arabia. Women were more prone to be obese than men. The prevalence of obesity is 14.2% among men and 23.6% among women (Al-Othaimen et al., 2007). Basically, the obesity risk depends on two important mutually-interacting factors: (1) genetic variants (single-nucleotide polymorphisms, haplotypes); (2) exposure to environmental risks (diet, physical activity etc. (Marti et al., 2008).

Genetic variants associated with obesity are being discovered using candidate gene approaches, genome-wide linkage studies, and genome-wide association studies. Identification of genes that contribute to risk of obesity will allow identifying individuals who are at risk, and it may lead to new therapies for treatment and prevention (Loos, 2009, Hebebrand et al., 2010) The search for genes that increase the susceptibility to develop obesity has

become important. One such candidate gene is the GNB gene. The G-protein $\beta 3$ subunit (GNB3) gene, which consists of 12 exons, is located on chromosome 12p13 and encodes the $\beta 3$ subunit of heterotrimeric G proteins (Wettschureck et al., 2005). Siffert *et al.*, 1998, were the first to describe the C825T (C-to-T substitution at nucleotide 825 in exon 10) (rs5443) polymorphism of the GNB3 gene. This polymorphism leads to a truncated splice variant (G $\beta 3$) in which the nucleotides 498–620 of exon 9 are deleted. The product of the *GNB3* 825T is 41 amino acids smaller than the wild-type 825C allele product. The *GNB3* 825T allele product has enhanced activation of heterotrimeric G proteins *in vitro* (Siffert et al., 1998). The *GNB3* 825T allele has been variably associated with obesity, hypertension, and atherosclerosis (Benjafeld et al., 2001, Hegele et al., 1999, Poch et al., 2002, Siffert, 2005). In this study we have demonstrated a correlation between the frequency of the T allele and obesity in a sample Saudi population.

2. Material and Methods

Sampling and calculation of BMI:

A total of 116 Healthy volunteers between 18-60 years old, both genders were involved in the study. Ethical committee approval was obtained and all volunteers signed a consent form to participate in the study. Individuals with chronic diseases such as diabetes mellitus, hypertension or thyroid dysfunction were excluded. The BMI was calculated from the height and weight of the volunteers and they were divided into various categories based on the WHO system of classification.

Molecular analyses:

Genomic DNA was extracted from whole blood using Qiagen DNA extraction kit and DNA was stored at -20°C for PCR analysis of the GNB3 gene. The PCR for the GNB gene was done using primers 5'-TGACCCACTTGCCACCCGTGC-3', 5'-GCAGCAGCCACCGCTGGC-3' and then subjected to PCR using a reaction mix (25µl consisting of 12.5µl of hot green master mix, 0.5µl of each primer to a final concentration of 0.1 µM, 7.5µl of DNA and 4.5µl of nuclease free water). Initial denaturation at 94°C for 5 min, denaturation at 94°C for 30 sec, annealing at 68°C for 30 sec, extension at 72°C for 30 sec and final extension at 72°C for 30 sec. The PCR was carried out for 40 cycles. PCR products were then subjected to restriction to generate the Restriction Fragment Length Polymorphism (RFLP) by restriction enzyme *Bsa*II for 30 min at 60°C incubation and inactivated for 20 min at 80°C. Samples were run in 3% agarose gel for 30 min with 70 volt current, and gel was pictured using gel documentation system and the band was calculated against 100 bp DNA ladder.

Statistical analyses:

Statistical analyses were done using the SPSS software to compute the p value as a measure of significance between the genotype and obesity of the subjects.

3. Results

Out of the 116 samples, 68.1% belonged to age group of 20s years, 23.3% were in the 30s, 3.4% in the 40s, 3.4% in 50s and 1.7% in 60s.

Body Mass Index (BMI):

The BMI was calculated from the height and weight of the volunteers and they were divided into various categories based on the WHO system of classification (table 1).

Table 1. Frequency of study subjects according to BMI classification

Category	BMI range	Frequency
Normal	18.5-25	55
Underweight	10-18.5	44
Border line obese	25-30	10
Obese	30-40	4
Over obese	40-60	4

Molecular characterization:

On genotyping the GNB3 gene, we get three different genotypes based on the RFLP patterns. PCR product of size 268 bp was obtained as shown in Figure 1.



Figure 1. A 3% (w/v) agarose gel showing the amplification of human GNB3 C825T gene by PCR.

Lane M: DNA marker

Lanes 1-13: PCR product of size 268 bp

The PCR products were restricted by using *Bsa*II to determine the presence or absence of the GNB3 C825T polymorphism. Alleles T represent the absence of restriction site while allele C indicate the presence of restriction site. The TT allele gave a 268-bp, the CC allele gave 153-bp and 116-bp fragments, and the heterozygous alleles gave all the three bands after the restriction enzyme digestion as shown in Figure 2.

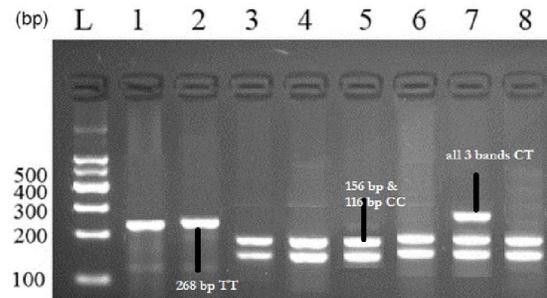


Figure 2. A 3% (w/v) agarose gel showing the digested PCR products for GNB3 C825T polymorphism genotyping.

Lane L: Marker

Lane 1: Uncut PCR product of 268 bp

Lane 2: TT genotype with 268 bp fragment

Lane 3,4,5,6,8: CC genotype with two fragments of sizes 156 bp and 116 bp

Lane 7: CT genotype with all 3 bands of sizes 268 bp, 156 bp and 116 bp

The distribution of the various genotypes amongst the individuals with relation to obesity status is shown in figures 3,4,5,6. We found that the presence of the T allele was a major contributing factor to obesity because from our study group we observed a higher occurrence of TT genotype in obese and overobese people while the normal people had a high occurrence of CT genotype and the underweight people had a high occurrence of CC genotype.

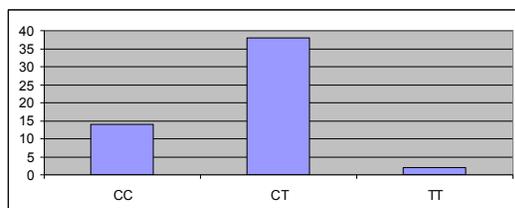


Figure 3. Distribution of genotypes in normal individuals

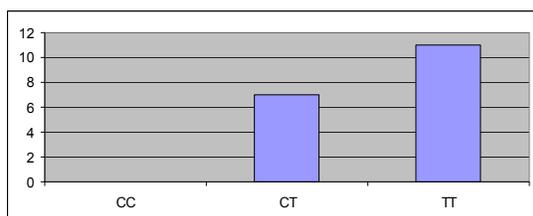


Figure 4. Distribution of genotypes in Borderline obese individuals

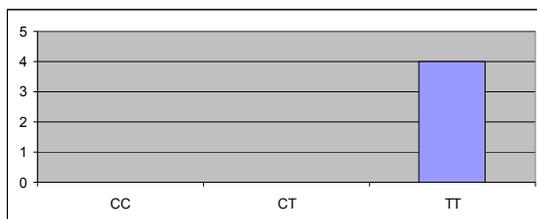


Figure 5. Distribution of genotypes in obese and overobese individuals

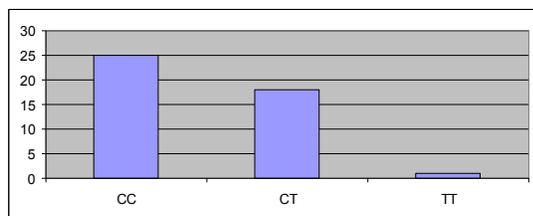


Figure 6. Distribution of genotypes in underweight individuals

Statistical analyses showed a high significance with p value of 0.000 between the presence of T allele and obesity in the population under study.

4. Discussions

In the present study, we have observed that the TT genotype and the T allele frequencies are more

frequent in obese compared to non obese subjects. The G-proteins are signal transducers that communicate information from hormones to intracellular signaling pathways (Siffert *et al.*, 1999, Nives *et al.*, 2001). The G-protein $\beta 3$ subunit (GNB3) gene, which consists of 12 exons, is located on chromosome 12p13 and encodes the $\beta 3$ subunit of heterotrimeric G proteins (Levine *et al.*, 1990). Siffert *et al.*, 1998 were the first to describe the C825T (cytosine -to-thymine substitution at nucleotide 825 in exon 10) (rs5443) polymorphism of the GNB3 gene. This polymorphism leads to a truncated splice variant (G $\beta 3$) in which the nucleotides 498–620 of exon 9 are deleted.

In most studies, the GNB3 825T allele increased the risk for hypertension (Nürnberg *et al.*, 2004) and related cardiovascular phenotypes (Jacobi *et al.*, 1999), and with obesity (Yamamoto *et al.*, 2004), psychological syndromes (Zill *et al.*, 2000, Kumugi *et al.*, 2002), type 1 diabetes complications (nephropathy, retinopathy and neuropathy) (Fogarti *et al.*, 1998, Shcherbak *et al.*, 2001), type 2 diabetes (Dzida *et al.*, 2002), cancer (Kiani *et al.*, 2005) and various immunological responses (Krippel *et al.*, 2004). However, evidence for relevance of GNB3 rs5443 to obesity is currently inconsistent. The T allele of GNB3 rs5443 SNP has been reported to predispose to obesity in German (Stefan *et al.*, 2004), Chinese and South African populations Siffert *et al.*, 1998). On the contrary, this association with obesity has not been replicated in white Danish subjects (Anderson *et al.*, 2006) and in a Japanese study (Hayakawa *et al.*, 2007).

A multi-ethnicity study done by Siffert *et al.*, 1999, showed that the GNB3 825T allele was associated with elevated body mass index regardless of ethnicity. The relative frequency of the 825T allele in that study was highest in black Africans (82%), intermediate in Chinese (47%), and lowest in Germans (32%). Siffert in 2001 suggested that this 825T allele distribution clearly reflects the human migration path from their cradle in East Africa to the Asian to the Western world. Particularly, it is interesting to find that the black Africans with highest 825T allele frequency are often prone to civilization diseases such as hypertension, obesity, and Type 2 diabetes when they change from their natural lifestyles to the typical Western sedentary one. In fact, Gutersohn *et al.*, 2000 showed that regular physical activity modulated the association between the GNB3 825T allele and body mass index in postpregnant women. The physiological role of GNB3 gene in the development of obesity is not fully understood, it is known that signal transduction alteration may modulate disease susceptibility (Su *et al.*, 1993). The current hypothesis is that the 825T

allele predisposes to obesity possibly via hyperinsulinemia (Hauner *et al.*, 2002), by Gai2 signal transduction (Su *et al.*, 1993) or a lower lipolytic response of adipose tissue to catecholamines (Ryder *et al.*, 2002)

On the basis of the available data, it is difficult to say whether the 825T allele exerts a co dominant or a recessive influence on BMI, the latter being a weak phenotype and a quantitative trait strongly influenced by environmental factors. In our study, we have strongly established a correlation between the T allele and obesity. Further investigations in this area are required to comprehensively define the role of the T allele in obesity. Our study with a limited sample size of 116 volunteers has given a clear indication that the T allele frequency is associated with obesity. We recommend that this study be extended to a GWAS (Genome wide association study) to enable us to convincingly document the extent of involvement of the T allele in obesity. In fact, the genotyping can be actually used to even predict the chances of obesity of people at a later stage in life.

Acknowledgements:

This project was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah under grant no. (562/130/1431). The authors, therefore, acknowledge with thanks DSR technical and financial support.

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7/1/2013