Genetic Polymorphisms in the Fat Mass and Obesity-Associated Gene Confers Risk of Obesity in Iraqi Population

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Abstract: Problem statement: Obesity is strongly influenced by genetic factors, with an estimated heritability of 60% BMI. Genetic susceptibility to the common form of obesity appears to be polygenic. Although theoretical analyses emphasized the power of genetic association study in common polygenic diseases, the search for genes conferring the risk of obesity has thus far not been very successful. Approach: In this research, DNA was extracted from 100 individuals who diagnosed as diabetes mellitus and have obesity referred to Al-Kindy research and therapeutic unit and in Baghdad 40 subject used as control. Thirty cycles of PCR were performed on exons 2 and 3 of the FSHß gene, which encode for the translated FSH ß protein. Results: For each exon, 30 cycles of PCR were performed at 95°C for 1 min, 55°C for 30sec and 72°C for 30 sec. Samples were subjected to sequencing and the results showed that the signals were poor and there is no capability to analyze so, we were recommended to do cloning the fragment of DNA to gain good signals. We did not observe significant association between rs9939609 and type 2 diabetes. Conclusion: Two SNPs (rs16952777 and rs1107355) in LD block 1 were nominally associated with type 2 diabetes. SNPs in the same block were also nominally associated with fasting glucose concentrations in no diabetic subjects (control subject). However, none of these associations remained significant after adjustment for multiple testing.

Key words: Same block, associations remained, remained significant, nominally associated, results showed, diabetic subjectsm, genetic association, genes conferring, estimated heritability

INTRODUCTION

Obesity is strongly influenced by genetic factors, with an estimated heritability of 60% BMI (El-Nabarawy et al., 2010; Souren et al., 2007). Genetic susceptibility to the common form of obesity appears to be polygenic. Although theoretical analyses emphasized the power of genetic association study in common polygenic diseases, the search for genes conferring the risk of obesity has thus far not been very successful. A few reported associations with genes such as GAD2, ENPP1 and INSIG2 also yielded inconsistent results in replication efforts (Boutin et al., 2003; Meyre et al., 2005; American Diabetes Association, 2011; Herbert et al., 2006). Recently, several independent studies using different approaches reported strong associations of genetic variants in the Fat mass and Obesity-Associated (FTO) gene with obesity in populations of European origin (Frayling et al., 2007; Dina et al., 2007).

Zeggini et al. (2007) initially found the association of FTO genetic variants with type 2 diabetes in a genome-wide association study for type 2 diabetes. However, the association was abolished by adjustment for BMI, indicating that the association with type 2 diabetes was mediated through an effect of obesity (Zeggini et al., 2007; Yuan et al., 2006). They replicated the associations (rs9939609) with obesity in a total of 38,759individuals (Frayling et al., 2007).

Dina et al. (2007) concurrently reported strong associations of single-nucleotide polymorphisms (SNPs) (rs1421085 and rs17817449) of the FTO gene with childhood and severe adult obesity.

Two other genome-wide association studies (Scuteri et al., 2007; Hinney et al., 2007) also independently reported the associations of nearby FTO genetic variants (rs9930506, rs8050136, rs7193144,
rs1121980 and rs9939973) with obesity and obesity-related traits in European and Hispanic populations. All these SNPs fall in a region of strong Linkage Disequilibrium (LD) in intron 1 of the FTO gene (Gibbs et al., 2003). The effect of FTO genetic variants on common obesity is also substantial in the European population.

Adults who are homozygous for the risk-conferring rs9939609 A allele weighed 3 kg more and had a 1.67-fold increased odds ratio of obesity when compared with those without a risk allele (Frayling et al., 2007; Barrett et al., 2005).

The calculated population-attributable risk is 22% for common obesity in populations of European origin (Frayling et al., 2007; Matthews et al., 1985). Reproducibility is essential for reported genetic associations, especially among populations of different ethnic backgrounds. However, studies in an Oceanic population (Ohashi et al., 2007), African Americans (Hinney et al., 2007), Han Chinese (Li et al., 2008; Shahwan-Akl, 2010) and Japanese (Horikoshi et al., 2008; Purcell et al., 2007) failed to detect associations between previously reported SNPs and obesity or obesity-related traits.

Although the limited sample size and power of these studies is the most likely reason for the lack of association, there is emerging evidence showing that other FTO SNPs not in LD with rs9939609 may be the causative variant in non-European populations (Grant et al., 2008).

This study introduces a new investigation of the association of FTO genetic variants with obesity and type 2 diabetes of Iraqi population. Instead of testing only a few variants, we used a gene based approach Neale and Sham (2004); Tabor et al. (2002) and Purcell et al. (2003) by selecting potentially functional and common SNPs from the 3’ end of the neighboring. RPGRIP1L gene to the 5’ flanking region of the FTO gene. Their associations with obesity-related quantitative metabolic traits were also analyzed. To examine the association of fat mass-and obesity associated (FTO) gene variant with obesity in Iraqi diabetic obese patients.

MATERIALS AND METHODS

Subject: One hundred and 6 subject individuals age range (15-50) years who participate in this study to fill from individuals questionnaire.

DNA analysis: DNA was extracted from 100 individuals who diagnosed as diabetes mellitus and have obesity refer to Al-Kindy research and therapeutic unit in Baghdad 40 subject used as control. Thirty cycles of PCR were performed as described previously exon 2 and 3 of the FSHß gene, which encode for the translated FSHß protein. For each exon, 30 cycles of PCR were performed at 95°C for 1 min, 55°C for 30sec and 72°C for 30 sec. The MgCl2 concentration was 2.0 mM for exon 2 and 2.5 mM for exon 3. Primer sequences include: Exon 2 sense (AGT TTC TAG TGG GCT TCA TTG TTG G) exon 2 antisense (TGG CTA AAG GAC TCA TGG CTG); exon 3 sense (GCT AAA TAG GAACCT TCA C) and exon 3 antisense (TAT GTG GCC TGA AAT GTC C). A negative control, containing all reagents except DNA, was included in each PCR. The PCR products were then electrophoresed on agarose gels, ethanol precipitated and subjected to dideoxy DNA sequencing using Big Dye Terminator Cycle (ABI Prism, PE Applied Bios stems, Foster City, CA).

Sequencing reactions were done using the ABI Big Dye Terminator kit (PE Applied Bios stems). Briefly, duplicate reactions of 20 ng template of the PCR products were amplified in a total reaction volume of 20 µL using either a forward or reverse primer. After the sequencing amplification, unincorporated nucleotides and primers were removed by spin column chromatography (Princeton Separations, Trenton, NJ) and dried in a vacuum centrifuge. Template suppression reagent was added to each of the samples, which were then vortexes briefly and denatured at 95°C for 2min.

Samples were immediately placed on ice for 2 min and vortexed again to mix. Then, the samples were analyzed on the ABI 310Automated DNA Sequencer (ABI Prism, PE Applied Bios stems).

Sequencing was confirmed in forward and reverse directions three times each for both probands and the parents.

Sequences analysis: Samples were subjected to sequencing (Bioneer, Korea) and the results showed that the signals were poor and there is no capability to analyze so, we were recommended to do cloning the fragment of DNA to gain good signals as shown in Fig 1-2.

RESULTS

Basic demographic data, SNP information and structure of LD. The baseline characteristics of participants are shown in Table 1 and 2 (Souren et al., 2007). Graphical representation of SNPs in relation to the exon-intron PCR product show a band within a 419-kb region containing the FTO gene using genotype data from the CHB and CEU HapMap samples.
Fig. 1: Fragment of DNA

Fig. 2: Fragment of DNA

Fig. 3: PCR product of (FTO) gene electrophoresis on agarose gel (2%), 45 min/70 volt

Table 1: BMI for individual participants

| No | BMI | No | BMI | No | BMI | No | BMI |
|----|-----|----|-----|----|-----|----|-----|
| 1  | 38.0| 27 | 26.6| 57 | 29.3| 83 | 29.0|
| 2  | 34.1| 28 | 26.3| 58 | 2.7 | 84 | 29.7|
| 3  | 31.4| 29 | 35.2| 59 | 27.8| 85 | 28.5|
| 4  | 27.8| 30 | 25.0| 60 | 30.3| 86 | 30.3|
| 5  | 33.3| 31 | 23.7| 61 | 25.8| 87 | 34.1|
| 6  | 20.1| 32 | 18.0| 62 | 30.0| 88 | 25.8|
| 7  | 29.0| 33 | 27.0| 63 | 28.6| 89 | 33.1|
| 8  | 31.4| 34 | 34.0| 64 | 28.0| 90 | 28.8|
| 9  | 23.0| 35 | 39.5| 65 | 44.7| 91 | 36.8|
| 10 | 24.0| 36 | 41.3| 66 | 30.0| 92 | 42.5|
| 11 | 27.5| 37 | 38.5| 67 | 27.3| 93 | 33.3|
| 12 | 22.8| 38 | 32.6| 68 | 34.0| 94 | 30.1|
| 13 | 24.1| 39 | 19.6| 69 | 95 | 25.4|
| 14 | 32.8| 40 | 19.6| 70 | 96 | 25.8|
| 15 | 30.0| 41 | 22.2| 71 | 97 | 34.8|
| 16 | 35.7| 42 | 28.8| 72 | 98 | 30.8|
| 17 | 27.4| 43 | 35.4| 73 | 99 | 32.9|
| 18 | 30.7| 44 | 35.1| 74 | 100| 25.9|
| 19 | 29.1| 45 | 26.7| 75 | 101| 27.3|
| 20 | 25.0| 50 | 42.0| 76 | 102| 21.5|
| 21 | 28.0| 51 | 31.8| 77 | 103| 28.0|
| 22 | 28.5| 52 | 32.2| 78 | 104| 26.3|
| 23 | 31.2| 53 | 36.8| 79 | 105| 30.9|
| 24 | 25.0| 54 | 26.4| 80 | 106| 32.0|
| 25 | 37.5| 55 | 28.3| 81 | 107| 30.3|
| 26 | 34.6| 56 | 27.8| 82 | 108| 25.8|

Table 2: The reaction was carried out as following:

| Step temperature | Time | no of cycles |
|------------------|------|--------------|
| Denaturation 1 first loop | 95°C | 3 min | 1 cycle |
| Denaturation 2 | 94°C | 1 min | 30 cycles |
| Annealing | 62°C | 1 min |
| Extension 1 | 72°C | 1 min |
| Extension 2 | 72°C | 5 min | 1 cycle |

Figure 3 shows association analysis of genetic variants of the FTO gene with obesity and BMI.

**DISCUSSION**

The rs9939609 A allele was identified as the risk variant for obesity in populations of European ancestry (Frayling et al., 2007). Among the 19 SNPs in this study, the rs9939609 A allele was strongly associated with obesity (P=7.0-10^{-4}). The per-a allele increase of odds ratio for obesity was 1.43 (95% CI 1.16-1.75). The association remained significant after correction for multiple testing. The genotypic odds ratio for obesity was 2.60 (1.24-5.46) (P=0.011) for the AA genotype and 1.32 (1.05-1.66) (P=0.018) for the AT genotype. The genetic model was best fit with an additive model (P=7.0-10^{-4}). 0.0098 and 0.014 for additive, recessive and dominant model, respectively). When different criteria for obesity were applied (16-24), the associations were also significant (allelic P=7.6-10^{-4} for obesity defined as BMI \_28 kg m^{-1} and 0.0081 for BMI 27 kg m^{-1}).
The frequency of the rs9939609 A allele (12.6%) was substantially lower in our study than that of European populations (45%) (Frayling et al., 2007), corresponding to a lower population-attributable risk fraction of 8.7% in the Chinese population. The rs9939609 A allele was also associated with increased BMI (P=0.0024) and weight (P=0.0065).

In our study cohort, carriers with AA genotype were heavier than those with TT genotypes (28.08 ± 8.45 kg/m²) (P=0.0088 and 0.048, respectively). Each additional copy of the rs9939609 a allele was associated with a BMI increase of a mean 0.124 Z score units, equivalent to -0.37 kg/m² for a person 1.7 m tall.

The extent of variance in BMI explained by rs9939609 was 0.5% in the Chinese population. There was no heterogeneity in the per-allele increase of odds ratio for obesity and effect size on BMI among different study populations. We did not detect any significant interaction between rs9939609 genotype and age or sex on the risk of obesity or BMI.

**Association analysis of genetic variants of the FTO gene with type 2 diabetes:** It not observes significant association between rs9939609 and type 2 diabetes. Two SNPs (rs16952777 and rs1107355) in LD block 1 were nominally associated with type 2 diabetes. SNPs in the same block were also nominally associated with fasting glucose concentrations in no diabetic subjects (control subject). However, none of these associations remained significant after adjustment for multiple testing.

**CONCLUSION**

According to the samples were tested, it can be concluded that there is no significant association between rs9939609 and type 2 diabetes. Two SNPs (rs16952777 and rs1107355) in LD block 1 were nominally associated with type 2 diabetes. SNPs in the same block were also nominally associated with fasting glucose concentrations in no diabetic subjects (control subject). However, none of these associations remained significant after adjustment for multiple testing.

Also, samples were subjected to sequencing showed that the signals were poor and there is no capability to analyze so, it is recommended to do cloning the fragment of DNA to gain good signals.
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