Association between exonic polymorphism (rs629849, Gly1619Arg) of IGF2R gene and obesity in Korean population

Seung-Ae Yang*

College of Nursing, Sungshin Women’s University, Seoul, Korea

The aim of this study is to investigate the relationship between single nucleotide polymorphisms (SNPs) and susceptibility to obesity. A previous study suggested that insulin-like growth factors (IGFs) may affect obesity and that IGFs regulate cellular signals by receptors that include the insulin-like growth factor 1 receptor (IGF1R) and the insulin-like growth factor 2 receptor (IGF2R). In this research, the rs3743262 and rs2229765 SNPs of IGF1R gene and rs629849 and rs1805075 SNPs of IGF2R gene were genotyped in 120 overweight and obese patients with a BMI ≥ 23 kg/m² (Body Mass Index) and 123 healthy controls with a BMI of 18.5-23.0 kg/m². Genotyping of each SNP was performed by direct sequencing. Among tested SNPs in IGF1R and IGF2R genes, rs629849 SNP of IGF2R gene showed significant association with obesity (OR = 1.86, 95% CI = 1.02-3.40, \( P = 0.044 \) in codominant model; OR = 1.99, 95% CI = 1.10-3.57, \( P = 0.020 \) in dominant model; OR = 1.93, 95% CI = 1.13-3.31, \( P = 0.013 \) in log-additive model). And allele distribution between the control group and overweight/obese group also showed significant difference (OR = 1.93, 95% CI = 1.14-3.28, \( P = 0.015 \)). In conclusion, these results indicate that rs629849 SNP of IGF2R might be contributed to development of obesity in the Korean population.

Keywords: Overweight, Obesity, IGF1R, IGF2R, Single nucleotide polymorphism

INTRODUCTION

Obesity is a growing medical problem in Korea (Lee et al., 2010; Mak et al., 2015; Paik et al., 2015). Obesity has been known to relate to many other diseases or bad conditions, such as cancer (Goday et al., 2015), stress (Mak et al., 2015), heavy alcohol drinking (Kim et al., 2014), and metabolic syndrome, which causes heart diseases (Byeon et al., 2015).

Many factors may affect the development of obesity, however, obesity shows complex relations of genetic and environmental factors (Apalasamy et al., 2015; Doo et al., 2015; Ghosh et al., 2014; Hruby et al., 2015). Many researchers have reported that various cytokines may influence the cellular signals affected by obesity (Kohlgruber et al., 2015; Wueest et al., 2015), among them, insulin-like growth factors (IGFs) are major axis linked to insulin and growth hormone (GH) (Coughlin et al., 2015; Savastano et al., 2014). They are major hormones affecting free fatty acid, plasma glucose, and adipose tissue, and that may show modified profiles in obesity (Oh et al., 2015). Additionally, IGF1 is released in liver by stimulation of GH, and IGF1 receptor signal may also affect insulin signal (Garwood et al., 2015), however, nutrition, exercise, stress, and body mass index (BMI) may affect the IGF and GH levels (Fontana et al., 2010; Glaser et al., 2010; Greer et al., 2011; Sherlock et al., 2007; Ubertini et al., 2008). Peet et al. reported IGF1 may be associated with beta-cell autoimmunity (Peet et al., 2015), and Salmon et al. reported that IGF1 level may be associated with obesity resistance (Salmon et al., 2015).

Above mentioned studies suggest that IGF may affect or be affected by obesity. IGFs may affect cellular signals through its receptors, the IGF1 receptor (IGF1R) and IGF2 receptor (IGF2R) (Kashyap, 2015; Zhu et al., 1997). IGF receptors have genetic variations that can affect various features in humans, such as cause
the SHORT syndrome (Prontera et al., 2015), growth restriction (Begemann et al., 2015), and cancer responses to the receptor antibody therapies (Cao et al., 2014; Lee et al., 2015). However, there was no study directly reported on whether or not obesity and IGF1R gene or IGF2R gene is associated. Therefore, the relationship between obesity and the single nucleotide polymorphisms (SNPs) of IGF1R gene and IGF2R gene were investigated in this study.

**MATERIALS AND METHODS**

**Study subjects**

In the present study, a total of 243 subjects were analyzed (Table 1). These subjects were recruited among participants that examined a general health check-up program. Subjects with severe diseases such as stroke, psychiatric disorders, and cancers were excluded. The biochemical characteristics of individuals were measured such as fasting plasma glucose, fasted glycated hemoglobin (HbA1c), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). Body mass index (BMI) is calculated as weight (in kilograms) divided by the square of height (in meters). According to the classification of Korean Society for the Study of Obesity (underweight, BMI < 18; normal, BMI 18 to < 23; moderately obese, BMI 23 to < 25; obesity I, BMI 25 to < 30; obesity II, BMI ≥ 30), subjects were divided into two subgroups, the abnormal (overweight/obese) group (BMI ≥ 23) and the normal group (18 < BMI < 23).

**SNP selection and genotyping**

Peripheral bloods of all subjects were collected in EDTA or heparin tube. Genomic DNAs were extracted by QIAamp® DNA mini kit (QIAGEN, Valencia, CA, USA). We selected exonic rs3743262 (Thr766Thr) and rs2229765 (Glu1043Glu) SNPs of IGF1R gene (Cho et al., 2012) and rs629849 (Gly1619Arg) and rs1805075 (Thr766Thr) and rs2229765 (Glu1043Glu) SNPs of IGF2R gene. Genotype of each SNP was determined by direct sequencing (MACROGEN, Seoul, Korea).

Polymerase chain reaction (PCR) was employed using the specific primers. Conditions of PCR were 35 cycles at 94°C for 30 sec, 58°C for 30 sec, and 72°C for 30 sec, and 1 cycle at 72°C for 5 min for the final extension reaction. SeqManII software (DNASTAR, Madison, WI, USA) was used to determine the genotype.

**Statistical analysis**

SNPStats (http://bioinfo.iconcologia.net/index.php) and SPSS 18.0 (SPSS Inc., Chicago, IL, USA) were used to determine the odds ratio (OR), 95% confidence interval (CI), and P-value. Multiple logistic regression models (codominant1, codominant2, dominant, recessive, and log-additive models) were applied and age and gender as covariables were adjusted. When the numbers of subject were below 5, the P-values were recalculated by Fisher’s exact test. The P-value below 0.05 was considered significant.

**RESULTS**

In the control group, the genotype distributions for all SNPs were in HWE [rs3743262 (P = 0.33) and rs2229765 (P = 0.45) SNPs of IGF1R gene and rs629849 (P = 1.00) and rs1805075 (P = 0.84) SNPs of IGF2R gene]. The genotype frequencies of the polymorphisms were compared between the control group and the overweight/obese group by using logistic regression models with adjustment for age and gender. The genotype distributions of rs3743262 and rs2229765 SNPs of IGF1R gene and rs629849 and rs1805075 SNPs of IGF2R gene in each group were shown in Table 2. Among tested SNPs in IGF1R and IGF2R genes, rs629849 SNP of IGF2R gene showed significant association with obesity [OR = 1.86, 95% CI = 1.02-3.40, P = 0.044 in codominant1 model (G/G genotype versus G/A genotype); OR = 1.93, 95% CI = 1.13-3.31, P = 0.013 in log-additive model (G/G genotype versus G/A genotype+a/A genotype), respectively. And allele distribution between the control group and overweight/obese group also showed significant difference (OR = 1.93, 95% CI = 1.14-3.28, P = 0.015). However, three SNPs did show any significant association with development of obesity.

In haplotype analysis, we analyzed haplotype using Haploview 4.2. There were four haplotypes (CG, TA, CA, and TG haplotype) in IGF1R gene and three haplotypes (GA, GG, and AA haplotype) in IGF2R gene. Among haplotypes, AA haplotype in IGF1R gene showed significant association with obesity [OR = 1.99, 95% CI = 1.10-3.57, P = 0.020 in dominant model (G/G genotype versus G/A genotype+a/A genotype); OR = 1.93, 95% CI = 1.13-3.31, P = 0.013 in log-additive model (G/G genotype versus G/A genotype versus A/A genotype), respectively. And allele distribution between the control group and overweight/obese group also showed significant difference (OR = 1.93, 95% CI = 1.14-3.28, P = 0.015). However, three SNPs did show any significant association with development of obesity.
The *IGF1R* gene showed significant difference between the control group and the overweight/obese group (*P* = 0.0138) (Table 3).

**DISCUSSION**

In the present study result, the two SNPs (rs3743262 and rs2229765) in *IGF1R* gene did not show any relationship with obesity, also, a SNP (rs1805075) in *IGF2R* gene did not showed any relationship. However, only one SNP (rs629849) of *IGF2R* gene was associated with obesity. Minor allele frequency (MAF) of rs629849 in our study was 0.1 in the control group. MAF of rs629849 in NCBI dbSNP (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=629849) was 0.0968. Therefore MAF in the control group is consistent with MAF in NCBI dbSNP. Significant associations are shown in the allele frequency, co-dominant model, dominant model, and log-additive model, respectively. In all of
them, ORs were positive in the minor allele (A). Therefore, it suggests that the minor allele “A” of the rs629849 in the IGF2R gene may be associated with development of obesity.

IGF2R is a multifunctional receptor that possesses binding sites for diverse ligands, including IGF2, retinoic acid, TGF-beta (TGBF1), urokinase-type plasminogen activator receptor (UPAR), and mannose-6-phosphate (M6P) (Killian et al., 1999). Additionally, IGF2R is involved in lysozyme reaction and cytotoxic T cell apoptosis (Kornfeld et al., 1989; Motyka et al., 2000). Which means IGF2R may have wide range of signal transduction, however, Le Stunff et al. reported that increased expression of mutated insulin(INS) and IGF2 gene may predispose offspring to postnatal fat deposition (Le Stunff et al., 2001). And Rodriguez et al. reported that certain haplotype composed by the gene region of IGF2-INS-thyroid hormone (TH) is related with obesity (Rodriguez et al., 2004). Also, IGF2-INS region is associated with past famine history in individuals (Tobi et al., 2009). Interestingly, IGF2R has degradation function of IGF2, which is known as mitogen (Yoon et al., 2012). Rezgui, studied the correlation of “A” allele and receptor function, however, the researchers concluded that it showed no direct effect and they suspected the linkage between other intronic polymorphisms.

In previous study, rs629849 of IGF2R gene was significantly associated with higher level of circulating IGF2 level in “A” allele homozygote woman (Hoyo et al., 2012). The authors concluded that “A” homozygote in rs629849 of IGF2R gene may modulate IGF2 level in sex-specific manner, and it may affect colorectal cancer risk as a mitogen effect of IGF2. Additionally, rs629849 of IGF2R gene was associated with advanced stage of oral cancer, that “GG” genotype showed ORs of 0.32 compared to “A” allele carriers (Yoon et al., 2012). The previous research results are, consistent with ours that the minor “A” allele may contribute to the obesity development.

In conclusion, though it was marginal relation in this study, we suggest that having “A” allele of rs629849 of IGF2R gene may be associated with development of obesity in the Korean population.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

REFERENCES

Apalasamy YD, Mohamed Z. Obesity and genomics: role of technology in unraveling the complex genetic architecture of obesity. Hum Genet 2015;134:361-374.

Begemann M, Zirn B, Santen G, Wirthgen E, Soellner L, Buttel HM, Schweizer R, van Workum W, Binder G, Eggermann T. Paternally inherited IGF2 mutation and growth restriction. N Engl J Med 2015;373:349-356.

Byeon CH, Kang KY, Kang SH, Bae EJ. Sarcopenia is associated with Framingham risk score in the Korean population: Korean National Health and Nutrition Examination Survey (KNHANES) 2010-2011. J Geriatr Cardiol 2015;12:366-372.

Cao Y, Roth M, Piperdi S, Montoya K, Sowers R, Rao P, Geller D, Houghton P, Kolf EA, Gill J, Gorlick R. Insulin-like growth factor 1 receptor and response to anti-IGF1R antibody therapy in osteosarcoma. PLoS One 2014;9:e106249.

Cho SH, Kim SK, Kwon E, Park HJ, Kwon KH, Chung JH. Polymorphism of IGF1R is associated with papillary thyroid carcinoma in a Korean population. J Interferon Ctokine Res 2012;32:401-406.

Coughlin SS, Smith SA. The insulin-like growth factor axis, adipokines, physical activity, and obesity in relation to breast cancer incidence and recurrence. Cancer Clin Oncol 2015;4:24-31.

Doo M, Kim Y. Obesity: interactions of genome and nutrients intake. Prev Nutr Food Sci 2015;20:1-7.

Fontana L, Klein S, Holloszy JO. Effects of long-term calorie restriction and endurance exercise on glucose tolerance, insulin action, and adipokine production. Age (Dordr) 2010;32:97-108.

Garwood CJ, Ratcliffe LE, Morgan SV, Simpson JE, Owens H, Vazquez-Villasenor I, Heath PR, Romero IA, Ince PG, Wharton SB. Insulin and IGF1 signalling pathways in human astrocytes in vitro and in vivo: characterisation, subcellular localisation and modulation of the receptors. Molecular Brain 2015;8:51.

Ghosh S, Murinova L, Trnovec T, Loffredo CA, Washington K, Mitra PS, Dutta SK. Biomarkers linking PCB exposure and obesity. Curr Pharm Biotechnol 2014;15:1058-1068.

Gläser S, Friedrich N, Ewert R, Schäper C, Krebs A, Dörr M, Völzhke H, Felix SB, Nauck M, Wallaschofski H, Koch B. Association of circulating IGF-I and IGFBP-3 concentrations and exercise capacity in healthy volunteers: results of the Study of Health in Pomerania. Growth Horm IGF Res 2010;20:404-410.

Goday A, Barneto I, García-Almeida JM, Blasco A, Lecube A, Grávalos C, Martínez de Icaya P, de Las Peñas R, Monereo S, Vázquez L, Palacio JE, Pérez-Segura P. Obesity as a risk factor in cancer: A national consensus of the Spanish Society for the Study of Obesity and the Spanish Society of Medical Oncology. Clin Transl Oncol 2015;17:763-771.

Greer KA, Hughes LM, Masternak MM. Connecting serum IGF-1, body size, and age in the domestic dog. Age (Dordr) 2011;33:475-483.
Hoyo C, Murphy SK, Schildkraut JM, Vidal AC, Skaar D, Millikan RC, Galanko J, Sandler RS, Jirtle R, Keku T. IGF2R genetic variants, circulating IGF2 concentrations and colon cancer risk in African Americans and Whites. Dis Markers 2012;32:133-141.

Hruby A, Hu FB. The Epidemiology of Obesity: A Big Picture. Pharcacoconomics 2015;33:673-689.

Kashyap MK. Role of insulin-like growth factor-binding proteins in the pathophysiology and tumorigenesis of gastroesophageal cancers. Tumour Biol 2015; Sep 14. [Epub ahead of print]

Killian JK, Jirtle RL. Genomic structure of the human M6P/IGF2 receptor. Mamm Genome 1999;10:74-77.

Kim HN, Song SW. Relationships of both heavy and binge alcohol drinking with unhealthy habits in Korean adults based on the KNHANES IV Data. Iran J Public Health 2014;43:579-589.

Köhler A, Lynch L. Adipose tissue inflammation in the pathogenesis of type 2 diabetes. Curr Diab Rep 2015;15:92.

Kornfeld S, Mellman I. The biogenesis of lysosomes. Annu Rev Cell Biol 1989;5:483-525.

Le Stunff C, Fallin D, Bougnères P. Paternal transmission of the very common class I INS VNTR alleles predisposes to childhood obesity. Nat Genet 2001;29:96-99.

Lee M, Jang Y, Kim K, Cho H, Jee SH, Park Y, Kim MK. Relationship between HDL3 subclasses and waist circumferences on the prevalence of metabolic syndrome: KMSRI-Seoul Study. Atherosclerosis 2010;213:288-293.

Lee Y, Wang Y, James M, Jeong JH, You M. Inhibition of IGF1R signaling abrogates resistance to afatinib (BIBW2992) in EGFR T790M mutant lung cancer cells. Mol Carcinog 2015; Jun 4. doi: 10.1002/mc.22342. [Epub ahead of print]

Mak KK, Kim DH, Leigh JP. Sociodemographic differences in the association between obesity and stress: A propensity Score-Matched Analysis from the Korean National Health and Nutrition Examination Survey (KNHANES). Nutr Cancer 2015;67:804-810.

Motyka B, Korbutt G, Pinkoski MJ, Hleibin JA, Caputo A, Helman M, Barry M, Shostak I, Sawchuk T, Holmes CF, Gauldie J, Bleackley RC. Mannose 6-phosphate/insulin-like growth factor II receptor is a death receptor for granzyme B during cytotoxic T cell-induced apoptosis. Cell 2000;103:491-500.

Oh YT, Tran D, Buchanan TA, Selseth ME, Youn JH. 0-Defensin RTD-1 improves insulin action and normalizes plasma glucose and FFA levels in diet-induced obese rats. Am J Physiol Endocrinol Metab 2015;309:E154-160.

Paik JS, Jang SK, Han KD, Kim SD, Park YM, Yang SW. Obesity as a potential risk factor for blepharoptosis: The Korea National Health and Nutrition Examination Survey 2008-2010. PLoS One 2015;10:e0131427.

Peet A, Hamalainen AM, Kool P, Ilenen J, Knip M, Tillmann V, Group DS. Circulating IGF1 and IGFBP3 in relation to the development of β-cell autoimmunity in young children. Eur J Endocrinol 2015;173:129-137.

Proterra P, Micale L, Verrotti A, Napolioni V, Stangoni G, Merla G. A New Homozygous IGFIR Variant defines a clinically recognizable incomplete dominant form of SHORT syndrome. Hum Mutat 2015;36(11):1043-1047.

Rodriguez S, Gaunt TR, O’Dell SD, Chen XH, Gu D, Hawe E, Miller GJ, Humphries SE, Day IN. Haplotype analyses of the IGF2-INS-TH gene cluster in relation to cardiovascular risk traits. Hum Mol Genet 2004;13:715-725.

Salmon AB, Lerner C, Ikano Y, Motch Perrine SM, McCarter R, Sell C. Altered metabolism and resistance to obesity in long-lived mice producing reduced levels of IGF-I. Am J Physiol Endocrinol Metab 2015;308:E545-553.

Savastano S, Di Somma C, Barrea L, Colao A. The complex relationship between obesity and the somatotropic axis: the long and winding road. Growth Horm IGF Res 2014;24:221-226.

Sherlock M, Toogood AA. Aging and the growth hormone/insulin like growth factor-I axis. Pituatory 2007;10:189-203.

Tobi EW, Lumey LH, Talens RP, Kremer D, Putter H, Stein AD, Slagboom PE, Heijmans BT. DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. Hum Mol Genet 2009;18:4046-4053.

Ubertini G, Grossi A, Colobianchi D, Fiori R, Brufani C, Bizzarri C, Giannone G, Rigamonti AE, Sartorio A, Muller EE, Cappa M. Young elite athletes of different sport disciplines present with an increase in pulsatile secretion of growth hormone compared with non-elite athletes and sedentary subjects. J Endocrinol Invest 2008;31:138-145.

Wueest S, Item F, Lucchini FC, Challia TD, Muller W, Bluher M, Konrad D. Mesenteric fat lipolysis mediates obesity-associated hepatic steatosis and insulin resistance. Diabetes 2015; Sep 17. pii: db150941. [Epub ahead of print].

Yoon AJ, Zavras AI, Chen MK, Lin CW, Yang SF. Association between Glu1619Arg polymorphism of IGF2R domain 11 (rs629849) and advanced stage of oral cancer. Med Oncol 2012;29:682-685.

Zhu J, Kahn CR. Analysis of a peptide hormone-receptor interaction in the yeast two-hybrid system. Proc Natl Acad Sci U S A 1997;94:13063-13068.