Brief Genetics Report

Variations in Peptide YY and Y2 Receptor Genes Are Associated With Severe Obesity in Pima Indian Men

Lijun Ma, P. Antonio Tataranni, Robert L. Hanson, Aniello M. Infante, Sayuko Kobes, Clifton Bogardus, and Leslie J. Baier

Peptide YY (PYY) and Y2 receptor (Y2R) may be important in the central regulation of body weight and food intake. To determine whether genetic variation in PYY and/or Y2R may contribute to morbid obesity in humans, these genes were sequenced in 83 extremely obese Pima Indians (BMI ≥50 kg/m²). Sequencing of PYY identified three single nucleotide polymorphisms (SNPs) in the untranslated region. Sequencing of the Y2R coding region identified one missense (Ala172Thr) substitution and two silent substitutions. Eight additional SNPs in the 5′ untranslated region of Y2R were identified from public databases. These SNPs were genotyped in 489 full-heritage adult Pimas (362 severely obese and 127 non-diabetic, nonobese subjects), who are not first-degree relatives, for association analysis. The PYY variants were not associated with obesity, whereas four variants from two haplotype blocks in Y2R were marginally associated (P = 0.054–0.067) with obesity. However, if the analysis was restricted to men (n = 167, 100 obese and 67 lean), the PYY variants and two SNPs in Y2R that were in complete linkage disequilibrium were significantly associated with severe obesity (P = 0.001 and P = 0.002, respectively). Our data suggest that the PYY-Y2R pathway may influence body weight through a sex-specific mechanism, but this finding requires confirmation in other populations. Diabetes 54:1598–1602, 2005

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From the Diabetes Molecular Genetics Section, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Department of Health and Human Services, Phoenix, Arizona.

Address correspondence and reprint requests to Leslie J. Baier, PhD, Diabetes Molecular Genetics Section, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, 4212 North 16th St., Phoenix, AZ 85016, E-mail: lbaier@phx.niddk.nih.gov.

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PYY, peptide YY; SNP, single nucleotide polymorphism; Y2R, Y2 receptor.

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Hardy-Weinberg equilibrium when analyzed in the case, control, and combined groups, respectively (Table 1). The allele frequency of these two SNPs did not differ between the case and control group (Table 3). However, since several obesity genes in animal models have sex-specific effects, the analysis was repeated in men and women separately. In the gender-restricted analysis, SNP1/2 and SNP3 were associated with severe obesity in men (n = 167, 100 obese and 67 lean; P = 0.001) using a logistic regression full model (Table 3).

The human Y2R gene (NCBI accession no. U36269) on chromosome 4q31 consists of two exons that span 4.5 kb. Three variants were detected by sequencing the coding region of Y2R in the same 83 extremely obese subjects described above (Table 1). A G/A polymorphism, predicting an alanine to threonine substitution, was identified at codon 172 (Ala172Thr; SNP9). Two additional variants, both C/T predicting a silent (isoleucine) substitution, were identified at codons 195 and 312 (SNP10 and SNP11, respectively). These three coding SNPs and eight additional variants (SNPs 1–8, across 34 kb) (Table 1), located in the 5′ untranslated region that was identified in the dbSNP (http://www.ncbi.nlm.nih.gov) and/or Celera databases (http://abassays.celera.com), were genotyped in the 489 case/control subjects. SNP10 and SNP11 (SNP10/11) were found to be in perfect genotypic concordance among the 489 case/control samples and therefore provided identical information. Genotypes of these 11 SNPs were in Hardy-Weinberg equilibrium in the case, control, and combined groups, except for SNP10/11, which deviated modestly in the control group (P = 0.04) (Table 1). SNPs 6, 7, and 10/11 in Y2R were marginally associated (P = 0.054–0.067) with severe obesity (Table 3). However, when the analysis was restricted by gender, SNP10/11 was significantly associated with severe obesity in men (P = 0.002) (Table 3).

To better understand the underlying genetic models for these associations, logistic regression analyses were also applied to the entire case/control group, and the gender-restricted groups in different genetic models (Table 3). The PYY variants (SNP1/2 and SNP3) appear to function under a dominant model, where men with the major allele (both homozygotes and heterozygotes) were more prevalent in the obese group, and men homozygous for the minor allele tended to be in the lean group (P = 0.001–0.002, odds ratio [OR] >3.5). Variants in Y2R (SNPs 3, 6, and 7 in 5′ region, SNPs 10 and 11 in exon 2) were associated with severe obesity under a recessive model (Table 3). The strongest association in men was with SNP10/11, where men homozygous for the C allele tended to be in the lean group, and men homozygous or heterozygous for the T allele tended to be more prevalent in the obese group (P = 0.001, 0.33 < OR < 0.4).

A statistically significant interaction of genotype with sex was observed for several of these SNPs in both genes (PYY SNP1/2 and SNP3, P = 0.001 in dominant model; Y2R...
SNP10/11, P = 0.018 in recessive model (online appendix Table 4 [available at http://diabetes.diabetesjournals.org]).

The genotypic information from 11 variants (SNPs 1–11) in Y2R was used to determine the haplotype structure of this gene (Fig. 1). Three haplotype blocks were identified. Y2R SNPs 2–4 represent the first haplotype block, SNPs 5–7 represent the second block, and SNPs 8–11 represent the third haplotype block. SNP9, the only coding missense substitution identified in Y2R, which was physically located in the third block, had a minor allele frequency that was too low for confident assignment to any haplotype block. In the association analysis, at least one variant in each of the three haplotype blocks of Y2R was modestly associated with severe obesity, but variants in the third haplotype block (SNP10/11) show a greater difference in men.

The finding of associations between variants in PYY and Y2R and severe obesity was unexpected in men only and may represent false-positive associations. However, a recent study by Hung et al. (11) on the PYY-Y2R pathway in British Caucasians also described an association between

![D-prime and Δ² matrix for 11 SNPs identified in Y2R gene. D' is defined as a measure of allelic association and Δ² as a measure of concordance (http://www.meb.ki.se/genestat/tl/). B: Haplotype structure and diversity of Y2R gene. Haplotype blocks and their frequencies were estimated using an accelerated EM algorithm. A, major allele; C, minor allele. Tag SNPs that designate a parsimonious haplotype for each block. Thick line, frequency ≥10%; thin line, frequency between 1 and 10%.](http://diabetes.diabetesjournals.org)
Y2R SNPs and human obesity with two SNPs identical to these significant SNPs detected in our current study (SNP10/11, rs1047214 [nt585C/T], and rs2880415 [nt936C/T]), and this association was only present in men. They found that men homozygous for the C allele of SNP rs1047214 had lower BMI than men homozygous or heterozygous for the T allele in British Caucasians (P = 0.017) (11), and rs2880415 was almost in complete genotypic concordance with rs1047214 (11). These findings are consistent with our results in Pima Indians. Therefore, it is possible that PYY or Y2R, or a different gene in the pathway that is activated by their interaction, is hormonally controlled. For example, NPY (a family member of PYY) expression is increased in male sheep in a testosterone-dependent manner (14), and testosterone also contributes to age-related changes of NPY expression (15). Since PYY belongs to the same family as NPY, it is possible that PYY also has a sex hormone-regulated element or other indirect hormone-controlled factor regulatory sites. Based on the sequence analysis of the PYY putative promoter by MatInspector (release professional 7.2.2, http://www.genomatix.de), PYY SNP1 (PYY-1746) is located in the anchor site of the ribonucleoprotein-associated zinc finger protein (MOK-2) binding region, and PYY SNP2 (PYY-1653) is located in the c-Myb/c-Myb core binding region. MOK-2 is preferentially expressed in testis tissue (16), providing a plausible mechanism for sex-specific expression of PYY. c-Myb is a transcription factor expressed in the hematopoietic system and the gastrointestinal tract that regulates the exquisite balance among cell division, differentiation, and survival (17). Androgen can influence the c-Myb expression level in mice (18); therefore, c-Myb binding could also potentially regulate PYY expression in a sex-dependent manner.

A third explanation for our association to be observed only in men is that more significant, female-specific obesity genes mask the contribution of PYY and Y2R to obesity in women. For example, female-specific effects on BMI and/or obesity-related traits have been reported for variants in several genes, including resistin (19), UCP3 (20), and FOX2 (21,22).

As the present association was detected in a case/control study of extremely discordant individuals, the significance of these findings on the population basis is not yet clear. Further genetic analysis of PYY and Y2R in other populations should help clarify the biological importance of these genes in the development of severe human obesity.

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