A PYY Q62P variant linked to human obesity

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Peptide YY (PYY) has been implicated in the control of food intake through functional studies in rodents and humans. To investigate whether genetic alterations within this gene result in abnormal weight in humans, we sequenced its coding exons and splice sites in a large cohort of extremely obese (n = 379; average body mass index (BMI), 49.0 kg/m\textsuperscript{2}) and lean (n = 378; average BMI, 19.5 kg/m\textsuperscript{2}) individuals. In total, three rare non-synonymous variants were identified, only one of which, PYY\textsubscript{Q62P}, exhibited familial segregation with body mass. Through serendipity, previous studies based on cell culture revealed this precise variant to have altered receptor-binding selectivity \textit{in vitro}. We further show, using mouse peptide injection experiments, that while the wild-type PYY peptide reduces food intake, the mutant PYY\textsubscript{62P} had an insignificant effect in reducing food intake \textit{in vivo}. Taken together, these results are the first to support that rare sequence variants within PYY can influence human susceptibility to obesity.

**INTRODUCTION**

The most convincing evidence for a genetic component for obesity comes from twin and adoption studies supporting that the genetic transmission of obesity is at least as important as the non-genetic factors (1). Using genetic approaches, a number of candidate genes for obesity have been identified and the importance of several of these genes was ascertained through genetically engineered mice (2). Overall, genes that may contribute to obesity susceptibility can be considered in three broad areas. These include genes that (i) regulate food intake (3); (ii) participate in adipogenesis (4); and (iii) influence energy expenditure including mitochondrial proton leak and adaptive thermogenesis (5).

In the category of food intake regulation, functional studies both in humans and rodents indicate a potentially important role for peptide YY (PYY) in decreasing food intake (6–8). Following food intake and in proportion to meal size, PYY is secreted into the blood stream from L-cells in the gastrointestinal tract in two forms, PYY\textsubscript{1–36} and PYY\textsubscript{3–36} (9), and binds to neuropeptide receptors within the brain to reduce food intake. PYY\textsubscript{3–36} was previously shown to bind \textit{in vitro} with highest affinity to the neuropeptide Y receptor Y2 (NPY2R) (10), an observation that was further established \textit{in vivo} using Npy2r-deficient mice, in which peripheral administration of PYY\textsubscript{3–36} failed to reduce food intake (7). In rodents, intravenous administration of PYY\textsubscript{3–36} led to a reduction in food intake (6,7,11) and in both obese and lean human subjects, PYY\textsubscript{3–36} infusion markedly decreased food intake (8). Combined, these studies support an important physiological role for PYY in the regulation of feeding behavior.

In humans, little is known of the effects of genetic variation in PYY on susceptibility to obesity. In one limited study, DNA sequence analysis of PYY and its receptor, NPY2R, in 83 extremely obese Pima Indians suggested that certain common variants may be associated with severe obesity in males (12). However, in a separate study performed in 101 individuals with severe early onset obesity, no association was found between common or rare nucleotide variants in PYY and obesity (13). To directly address the effects of variations in the coding sequences of PYY on human body weight, we sequenced a large cohort of subjects at the two extremes of BMI.

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RESULTS

We sequenced the human PYY (NCBI accession no. NM_004160) coding exons and their splice sites in 379 obese and 378 lean individuals with a mean BMI of 49.0 and 19.5 kg/m², respectively. Sequence analysis revealed no significant frequency differences between two of the three common variant (>1% allele frequency) genotypes in the obese versus lean population (Table 1). For R72T, we observed a marginally significant (P-value = 0.02; χ² test) genotype frequency difference between our two cohorts, though this variant has been previously reported to lack association with BMI when tested in 952 Caucasians (13), which are of similar ethnicity as our population. In addition, three rare (<1% allele frequency) non-synonymous variants unique to either the obese or lean population were discovered (Table 1). Two variants, P9H and Q62P, were identified in two isolated obese individuals, whereas a nonsense variant, Y49/C3, was found in a single lean individual.

Based on the severe nature of the Y49/C3 nonsense substitution, we assessed this variant for segregation in the proband’s available extended pedigree. However, we found no relationship between this variant and BMI in 952 Caucasians (13), which are of similar ethnicity as our population. In addition, three rare (<1% allele frequency) non-synonymous variants unique to either the obese or lean population were discovered (Table 1). Two variants, P9H and Q62P, were identified in two isolated obese individuals, whereas a nonsense variant, Y49/C3, was found in a single lean individual.

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Table 1. PYY variants identified through resequencing

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*Genotype and variant numbering are based on NCBI accession no. NM_004160.

Figure 1. PYY Q62P. (A) Familial segregation of PYY Q62P (the arrow indicates the individual sequenced in the cohort). Black-shaded individuals in the pedigree are carriers of the 62P variant, while the lone gray-shaded individual is inferred to be an obligate carrier. (B) Clustal-W (24) and boxshade protein alignment analysis of the secreted PYY paralogs, human NPY and PPY. Amino acids PYY Q62/Q34 and PPY P63/P36 are marked with black arrows.

Familial segregation analysis of the obese variants showed no significant correlation between variant P9H and BMI in the small kindred that was available for genotyping (Supplementary Material Fig. S1B). On the other hand, PYY Q62P was found in four individuals in an extended family (Fig. 1A), and these had an average BMI adjusted for age and sex at the 87th percentile (including a 4-year-old child with congenital heart disease, which may restrict an obesity phenotype) compared with an average BMI at the 52nd percentile for five non-carriers (P = 0.0158, two-tailed t-test with Welch correction). In terms of an obesity phenotype, the Q62P proband was hypertensive but normolipidaemic as well as euglycaemic, and in comparison with other age, sex and BMI-matched subjects, lost weight quickly on a diet.
Matched controls on the effect of substituting PYY1–36 Q34 to a proline (10). This observation of a paralogous protein paralogs, whereas in PPY this residue is substituted by a extremely evolutionarily conserved both in PYY and NPY Q62, which represents Q34 in the secreted form of PYY, is intake primarily through rodent-based studies. Here, we PYY has been functionally implicated in the control of food intake secondary to hyperphagia rather than low energy expenditure. These data were suggestive of a possible role for PYY Q62P in influencing obesity susceptibility and encouraged further functional studies on its protein product. Serendipitously, the exact Q62P amino acid change had been previously studied in vitro (15). PYY amino acid position Q62, which represents Q34 in the secreted form of PYY, is extremely evolutionarily conserved both in PYY and NPY paralogs, whereas in PYY this residue is substituted by a proline (Fig. 1B). This observation of a paralogous protein amino acid change led several groups to conduct experiments on the effect of substituting PYY1–36 Q34 to a proline (10). Using nuclear magnetic resonance (NMR) and circular dichroism spectroscopy (CD spectra) studies as well as numerous cell culture assays, a profound functional consequence of this change, resulting in an altered binding affinity and specificity to several NPY receptors, was documented (10). Combined, our limited family segregation data and these previously established in vitro properties of PYY Q62P led us to test the effects of this variant on food intake in vivo.

Based on previous publications, implicating PYY3–36 as the major PYY peptide to reduce food intake in rodents (6), we generated a mutant PYY3–36 P34 peptide and sought to determine its effects on food intake in mice. Following a previously described protocol (11), we injected wild-type (Q34) and mutant (P34) PYY peptide into mice after a 24-h fast. We found that 6 h post-injection, the wild-type peptide significantly decreased food intake when compared with saline control as previously described (11). In contrast, the mutant peptide had no significant effect on food consumption when compared with saline control (P = 0.26, one-way ANOVA) (Fig. 2). In addition, we co-injected both the wild-type and mutant peptide and observed a consequence similar to mutant P34 alone (Fig. 2). This abolishment of wild-type peptide feeding inhibition in the co-injection experiments suggests that P34 has a gain of function mechanism of action thereby overcoming the wild-type peptide effect. Together, these results support a functional role for the PYY Q62P variant on the regulation of food intake.

**DISCUSSION**

PYY has been functionally implicated in the control of food intake primarily through rodent-based studies. Here, we explored the potential genetic contribution of this gene to human body weight through DNA resequencing in two extreme populations followed by familial and functional investigation. Based on our relatively large sample size, and previous reports (12,13), it appears that rare non-synonymous changes in this gene in the obese population are infrequent and their contribution to widespread weight differences in humans is likely to be minor. In addition, while our analysis was not designed as an exhaustive genetic association study between common variants in the PYY genomic region and BMI, we found three common variants, one of which showed marginal frequency differences between the obese and lean groups (Table 1). Whether other common non-coding variants in the extended genomic region may more strongly impact on human BMI remains to be further explored. Nevertheless, the finding of PYY Q62P variant and the demonstration of its functional importance suggest that a small subset of variants in PYY might be detrimental and potentially important in the etiology of weight differences.

Mechanistically, we can speculate on how the 62P variant might lead to a change in PYY function. Previous in vitro studies carried out using a mutant PYY1–36 P34 peptide demonstrated that this residue is of importance in terms of the binding specificity to other NPY receptors, specifically NPY1R (16,17), NPY4R (18) and NPY5R (19). As NPY2R ligand binding is predicted to result in appetite suppression, and NPY1R and NPY5R [also possibly NPY4R (20)] in appetite induction [reviewed in (21)], it is possible that the P62 variant shifts PYY function in vivo. This could be either through P62 activation of NPY1R and NPY5R (and/or NPY4R) and/or its competitive blockage of their natural peptide ligands, thus leading to a gain of function effect. We should point out that since our functional analysis was done in mice and not in humans, and previous receptor-binding assays were carried out in vitro, our functional data can only be suggestive regarding the causative nature of this variant in human obesity.
Finally, the finding of a nonsense variant, Y49*, in a lean individual and several of his lean to normal weight family members indicates that PYY haplo-insufficiency in humans does not promote obesity. In addition, the observation of decreased levels of fasting and post-prandial plasma PYY\textsubscript{3–36} levels in Y49* carriers versus controls support the notion that subtle changes in PYY levels are unlikely to profoundly influence human weight. Taken together, these data suggests that rare genetic changes in PYY may influence isolated cases of human obesity most likely through a gain of function mutation mechanism. Further mutation screening in PYY in large obese cohorts will provide a better estimate of the prevalence of such nucleotide changes and their effects on human body weight.

MATERIALS AND METHODS

Subjects
Obese Caucasian subjects were recruited from the patient population of the University of Ottawa Weight Management Clinic and the Heart Institute Lipid Clinic. Criteria for inclusion included a BMI >36 kg/m\textsuperscript{2} and a history of obesity for at least 10 years of adult life. Exclusion criteria included treatment with oral glucocorticoids, anti-psychotics, lithium or medical conditions including major depression, bipolar affective disorder or psychosis. Lean subjects of the same ethnic background were recruited from the Ottawa community. BMI for obese and lean subjects was categorized according to the population percentiles for age and sex using the Canadian Heart Health Survey data for subjects over the age of 18 years (data on file; Health Canada) and NHANES data for children (22). Inclusion criteria for the lean subjects include a BMI less than or equal to the 10th percentile for age and sex, with no prior history of having had a BMI greater than 25th percentile for age and sex for more than a 2-year consecutive period. Exclusion criteria include medical conditions affecting weight gain including hyperthyroidism, anorexia nervosa, bulimia, major depression or malabsorption syndromes. This study was approved by the institutional review boards of the University of Ottawa Heart Institute and the Ottawa Hospital and informed written consent was obtained from all participants. Genomic DNA was extracted from white blood cells by standard methods (23) or from saliva samples collected in Oragene Kits (DNA Genotek).

Sequencing and data analysis
Primers were designed to give a maximum product size of 500 bp and a minimum of 40 bp flanking the splice sites using the Exon Locator and eXtractor for Resequencing program (EXLR) (http://mutation.swmed.edu/ex-lax/). An M13 F tag (gttttccagtcacgacgttgta) and M13R tag (aggaaacagctatgaccat) was added to forward and reverse primers, respectively. Ten nanograms of DNA from each sample was amplified in a 10 \mu l PCR reaction using AmpliTaq Gold\textsuperscript{®} (Applied Biosystems) and cleaned using Tetra-ethylene glycol (TET) (http://www.jgi.doe.gov/sequencing/protocols/BETcleanup.doc). Sequencing reactions were performed using the M13 primers along with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) (http://www.jgi.doe.gov/sequencing/protocols/BigDye3.1auto1.0.doc) and cleaned again with TET before separation on a 3730xl DNA Analyzer (ABI). Base calling, quality assessment and assembly were carried out using the Phred, Phrap, Polynphred, Consed software suite (www.phrap.org). All sequence variants identified were verified by manual inspection of the chromatograms and by a second independent sequencing reaction.

Mouse peptide experiments
Two-month old 129/Sv male mice were individually housed in cages for 2 weeks prior to experimentation. Mice were housed in a temperature-controlled room under a 12-h light and dark cycle, given free access to water and fed ad libitum on a standard chow. After the 2-week period, the mice were fasted for 24 h and then injected intraperitoneally with 10 \mu g per 100 g of body weight PYY\textsubscript{3–36}, PYY\textsubscript{3–36} P34 (both from Bachem), or saline at the onset of the dark cycle (18:00). When both PYY\textsubscript{3–36} and PYY\textsubscript{3–36} P34 were injected, equal amounts were adjusted and combined so that they would give 10 \mu g per 100 g of body weight. Food intake was measured at 6, 24 and 48 h following injection by measuring the pre-weighed portions of food dispensed from wire cage tops. Cages were carefully monitored for evidence of food spillage or grinding, which were negligible. Experiments were performed in duplicate with an average of eight mice per study.

SUPPLEMENTARY MATERIAL
Supplementary material is available at HMG Online.

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Conflict of Interest statement. None declared.

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