Abstract: OBJECTIVE: The neuropeptide-Y (NP-Y) gene is a strong candidate gene in the pathophysiology of obesity-linked behavior, and several single-nucleotide polymorphisms of NP-Y have already been linked to body weight and appetite. However, the results from current studies remain inconclusive. The aim of the present study was to test whether a certain functional genetic variant (SNP rs16147) in the NP-Y promoter gene is associated with serum leptin levels and body fat distribution. METHOD: We genotyped and measured the serum leptin levels of the NP-Y rs16147 polymorphism in 1,097 Caucasian subjects in the context of a population-based, case-control multicenter study. We measured weight, height and waist circumference, from which we then calculated BMI and waist-to-hip ratio (WHR). RESULTS: We found the CT-genotype of the SNP rs16147 to be significantly associated with lower WHRs and higher serum leptin levels in women, compared to homozygote gene carriers. No association between rs16147, WHR and serum leptin levels was found in men. CONCLUSION: Our results provide evidence that the functionally relevant SNP in the NP-Y promoter gene affects body fat distribution and serum leptin levels in women, pointing towards possible behavioral effects of NPY in obesity.

DOI: https://doi.org/10.1159/000346799

Posted at the Zurich Open Repository and Archive, University of Zurich
ZORA URL: https://doi.org/10.5167/uzh-89317
Accepted Version

Originally published at:
Mutschler, Jochen; Abbruzzese, Elvira; Wiedemann, Klaus; von der Goltz, Christoph; Dinter, Christina; Mobascher, Arian; Thiele, Holger; Diaz-Lacava, Amalía; Dahmen, Norbert; Gallinat, Jürgen; Majic, Tomislav; Petrovsky, Nadine; Thuerauf, Norbert; Kornhuber, Johannes; Gründer, Gerhard; Rademacher, Lena; Brinkmeyer, Jürgen; Wienker, Thomas; Wagner, Michael; Winterer, Georg; Kiefer, Falk (2013). Functional polymorphism in the neuropeptide Y gene promoter (rs16147) is associated with serum leptin levels and waist-hip ratio in women. Annals of Nutrition Metabolism, 62(4):271-276.
DOI: https://doi.org/10.1159/000346799
Functional polymorphism in the neuropeptide Y gene promoter (rs16147) is associated with leptin levels and waist-hip ratio in women

Jochen Mutschler1,2†, Elvira Abbruzzese2†, Klaus Wiedemann3, Christoph von der Goltz1, Christina Dinter1, Arian Mobascher4, Holger Thiele5, Amalia Diaz-Lacava5, Norbert Dahmen4, Jürgen Gallinat7, Tomislav Majic7, Nadine Petrovsky8, Norbert Thierauf9, Johannes Kornhuber9, Gerhard Gründer10, Lena Rademacher10, Juergen Brinkmeyer11, Thomas Wienker6, Michael Wagner12, Georg Winterer5, Falk Kiefer1

†These authors contributed equally to this work.

1Department of Addictive Behavior and Addiction Medicine, Central Institute of Mental Health, University of Heidelberg, Square J 5 68159 Mannheim, Germany
E-mail: jochen.mutschler@puk.zh.ch

2Department of General and Social Psychiatry, Psychiatric University Hospital, Zurich, Militärstrasse 8, P.O. Box 1930, 8021 Zurich, Switzerland

3Department of Psychiatry, University of Hamburg, Germany

4Department of Psychiatry, Johannes Gutenberg University, Mainz, Germany

5Cologne Center for Genomics (CCG), University of Cologne, Germany

6Institute of Medical Biometry, Informatics and Epidemiology, University of Bonn, Germany

7Department of Psychiatry, Charité University Hospital, Berlin, Germany

8Institute of Psychology, University of Bonn, Germany

9Department of Psychiatry, Friedrich Alexander University of Erlangen Nuremberg, Germany

10Department of Psychiatry and Psychotherapy, Medical Faculty, RWTH Aachen University, Aachen, Germany

11Department of Psychiatry, Heinrich Heine University, Düsseldorf, Germany
Role of funding source: Funding for this study was provided within the framework of the German Priority Program SPP1226 "Nicotine – Molecular and Physiological Mechanisms in CNS" by grants from the German Research Foundation (DFG KI 782/5-1 and Wi1316/9-1).

Conflict of interest: All authors deny any conflict of interest.

Acknowledgments: We would like to thank Carsten Wied for his assistance with the data collection.

**Keywords:** NP-Y, Polymorphism, Obesity, Waist-hip ratio, Genetics, rs16147, Leptin

**Running head:** NP-Y gene variant is associated with waist-hip ratio
Abstract

Objective: The Neuropeptide-Y (NP-Y) gene is a strong candidate gene in the pathophysiology of obesity-linked behavior, and several single-nucleotide polymorphisms of NP-Y have already been linked to body weight, and appetite. However, the results from current studies remain inconclusive. The aim of the present study was to test whether a certain functional genetic variant (SNP rs16147) in the NP-Y promoter gene is associated with leptin and body-fat distribution.

Method: We genotyped and measured the serum leptin levels of the NP-Y rs16147 polymorphism in 1097 Caucasian subjects in the context of a population-based, case-control multicenter study. We measured weight, height and waist circumference, from which we then calculated body mass index and waist-hip ratio (WHR).

Results: We found the CT-genotype of the SNP rs16147 to be significantly associated with lower waist-hip ratios and higher serum leptin levels in women, compared to homozygote gene carriers. No association between rs16147, WHR and leptin levels was found in men.

Conclusion: Our results provide evidence that the functionally relevant SNP in the NP-Y promoter gene affects body-fat distribution and leptin plasma concentration in women pointing towards possible behavioral effects of NPY in obesity.
INTRODUCTION

Obesity is a major health problem with extensive consequences not only for those it affects but also for society as a whole [1]. In particular, central obesity – which is measured using waist-to-hip ratio (WHR) – is associated with increased risk of diabetes, depression, kidney disease, obesity-related cancers, and death from cardiovascular disease [2-5]. However, the mechanisms and pathways underlying central obesity and regulation of body-fat distribution are not completely understood. While the genetic risk factors [6] as well as the environmental factors [7-10] underlying obesity are still relative poorly understood, a better understanding of the complex interactions between physical, endocrinological, genetic and molecular phenotypes is needed [11]. Recent evidence suggest that only around 50 % of the variation between individuals in body weight has a genetic basis, but these effects are dominated by polygenetic environmental interactions that reflect many genetic influences affecting spontaneous physical activity, metabolic rate, endocrinological changes, and appetite behavior [1]. There are a large number of hormones and neuropeptides involved in regulating the homeostasis of body weight, appetite, and food intake. Current research has shown the leptin and neuropeptide-Y systems to be two of the most interesting and important regulators of appetite and energy homeostasis [12,13]. Since leptin, an anorexigenic peptide, is secreted by adipocytes in proportion to lipid reserves, leptin levels provide feedback about the body's fat stores [14]. Leptin is also involved in the long-term regulation of adiposity, and recent research has suggested that leptin diminishes food intake by signaling satiety in the hypothalamus [14]. In obese persons, a leptin-resistance mechanism affects leptin's regulatory effect, which explains the positive correlation observed between leptin levels and body-fat mass [15]. In contrast to leptin, neuropeptide-Y is an orexigenic neuropeptide, and it promotes food intake and helps reduce energy expenditure [16]. A recently published study found that changes of plasma levels of Anti-neuropeptide-Y plasma immunoglobulins are relevant to altered appetite and body weight in patients with depressive disorder [17]. Findings from a recent study suggest that circulating leptin directly engages NP-Y neurons, thus regulating body-weight homeostasis [18]. Furthermore, melatonin recently has been found to play a role in central appetite regulation by modulating the gene transcription of leptin and NP-Y [19]. Additionally, there is growing evidence that in addition to their roles regulating food intake and energy expenditure, both peptides (NP-Y and leptin) also closely interact with mesolimbic reward pathways [20,21]. Since the effects of food intake are self-reinforcing, there has been increased discussion recently as to whether severe obesity and addiction disorders share any common neuronal circuits.
Furthermore, gene variations in both systems (NP-Y and leptin) have been found to be associated with central obesity as well as numerous psychiatric conditions [14,23-25]. Obesity is to an essential degree consequence and measure of maladaptive eating behavior. Despite neurobiological processes controlling food intake, the interaction of genetic, psychological and endocrinological factors which thus constitute risk factors of weight gain and obesity are complex, but important to investigate [1]. Analysis of the candidate genes that regulate psychological and endocrinological pathways could lead to a better understanding of the pathophysiology of central obesity. To this end, we analyzed whether or not a certain functional genetic variant in the NP-Y-promoter gene (SNP rs16147) is associated with body-fat distribution and peripheral leptin levels in an ethnically homogenous sample of healthy white subjects.

Methods

Data Collection

For the case-control association analysis, we analyzed data from a cohort, investigating the phenotypes and genetics of nicotine dependence. Of our subjects, 466 were male, 631 were female (entire sample n=1097), and all were genotyped. Data were collected at seven recruitment centers located throughout Germany (Depts. of Psychiatry at the Universities of Aachen, Berlin, Bonn, Duesseldorf, Erlangen, Mainz, and Mannheim) between 2007–2009. All participants were required to be of German origin and to speak German at a native-speaker level. Only ethnically German subjects were included. Details on the recruitment, testing procedures, inclusion/exclusion criteria, and characterization of this multicenter study on nicotine dependence and smoking-related behavior have been published elsewhere [26,27].

Prospective subjects’ conformity to inclusion/exclusion criteria was assessed using a medical examination, a standardized psychiatric interview (SCID-I), questionnaires, drug screenings, alcohol testing, and CO measurement.

The present study was approved by the ethics committees of all participating centers; subjects were included in the study only after they had given written, informed consent.
Measures and testing procedures

The testing sessions were subjected to a strict timetable and included a standardized 600 kcal meal during a one-hour lunch break taken at noon. The assessments started at 8:30 am and lasted until 4:30 pm for all participants. The absence of psychiatric axis-I co-morbidity was verified using the Structured Clinical Interview, which is based on DSM-IV criteria (American Psychiatric Association, 2000). Body weight, body-mass index and waist-hip ratio were assessed for all participating subjects according to standard procedures. BMI was calculated as body weight (kg) divided by the square of height (m).

DNA preparation and genotyping

Genotyping was performed at the Cologne Center for Genomics (CCG) at the University of Cologne. DNA from fresh frozen EDTA blood was prepared using a Qiagen FlexiGene DNA Kit according to the manufacturer's instructions and normalized based on RNase P copy number measurement using the TaqMan RNase P assay from Applied Biosystems®, Foster City, Calif., USA. The SNP rs16147, a functional NP-Y promoter variant, was chosen to cover the functional expression of brain NP-Y. Genotyping was performed using SNPstream SNP genotyping assays. Genotyping call rates were 99%. All laboratory procedures were carried out blind to case control-status.

Hormonal measures

Blood samples were obtained between 2:00 and 4:30 pm by venipuncture, and were then anticoagulated with sodium EDTA (1 mg/ml whole blood) and immediately cooled on ice. Plasma was separated by centrifugation with 4000 g, and aliquots were frozen immediately and stored at -80°C until analysis (max. 6 months). The leptin analyses were performed at the Neurobiological Laboratory of the Department of Psychiatry at the University Hospital of Hamburg.
To measure leptin, we used a human leptin radioimmunoassay kit (Linco, St. Charlex, MO, USA). The detection limit was 0.25 ng/ml of plasma; intra- and inter-assay coefficients of variation for 4.9 and 15.7 ng/ml levels were below 8.5%.

**Data analysis**

Associations between genotype and gender as well as obesity and gender were evaluated using the chi-squared test. The gender-specific risk of obesity is given in odds ratio and its confidence interval. Due to the fact all three dependent variables (BMI, WHR and leptin) deviated from a normal distribution (Kolmogorov-Smirnov test for all three variables: p<0.001), we performed non-parametric, Mann-Whitney tests in order to compare the influence of the genotype. The genotypes were divided into two groups: one for homozygotes (CC and TT) and another for heterozygotes (CT). Additionally, we calculated correlative associations using Pearson correlations (two-tailed). All tested statistics indicating a p-value of 0.05 or less were considered significant. We tested for deviation from Hardy-Weinberg Equilibrium using a Fisher’s exact test. The data analysis was performed using SPSS Statistics, version 19.

**Results**

**Group characteristics**

A total of 1097 subjects were genotyped for SNP rs16147. This total sample was primarily female (631 female, 466 male), aged 34.7 ± 12.8 years. SNP rs16147 did not deviate from Hardy-Weinberg equilibrium (TT=258, 23.5%, CT=561, 51.1%, and CC=278, 25.3%; p=0.469). Genotype distributions for the study population (women and men) are presented in Table 1. The study population’s sociodemographic and clinical characteristics have been described in previous papers [27-29]. For the purposes of this study, obesity was defined as a body-mass index (BMI) of 30 or greater. Of the participants, n=120 have a BMI greater than 30 (10.9% of the sample); broken down by gender, n=57 of the women were obese (9%) compared to n=63 of the men (13.5%). Obesity was unequally
distributed between men and women (Chi²=5.578, p=0.018), with men having a higher probability of being obese compared to woman (OR=1.577, CI 1.08-2.31).

The mean WHR for homozygote allele carriers was 0.834 (± 0.955) compared to 0.819 (± 0.909) for heterozygote allele carriers. The TT and CC genotypes of rs16147 were significantly associated with an increased risk of higher waist-hip ratios in comparison to the CT genotype (Mann-Whitney U=108.760, p=0.008). This association seems to be gender specific, with homozygote (TT and CC; mean WHR=0.795 ± 0.083) women have significantly higher WHR than heterozygote (CT; mean WHR=0.773 ± 0.068) women (Mann-Whitney U=33.730, p=0.001). In men this comparison is not significant (Mann-Whitney U=22.424, p=0.264). However, comparisons of CC and TT alleles did not show any significant differences (Mann-Whitney U=5.877, p=0.728).

The mean BMI in women was 23.481 (± 4.855), and in men it was 25.301 (± 4.210). This difference was found to be significant (Mann-Whitney U=187,575, p<0.001). However, the mean BMI’s of homozygote allele carriers (24.386 ± 4.578) and heterozygote allele carriers (24.170 ± 4.805) did not differ significantly (Mann-Whitney U=140.927, p=0.212).

The mean leptin level in homozygote allele carriers was 8.798 ± 8.488 ng/ml, compared to 9.669 ± 9.155 ng/ml in heterozygote allele carriers, which indicates significantly higher leptin levels in heterozygote allele carriers (Mann-Whitney U=152,908, p=0.025). We found the highest leptin levels in heterozygote women (12.516 ± 10.091 ng/ml), which were higher than both homozygote women (11.626 ± 8.695 ng/ml), and homozygote men (homozygote: 5.509 ± 6.928 ng/ml; heterozygote: 5.05 ± 4.50725 ng/ml). Peripheral leptin levels were 12.115 (± 9.491) ng/ml in women, compared to 5.296 (± 5.921) ng/ml in men (Mann-Whitney U=55,668, p<0.001).

Finally, we conducted a serie of correlation analyses, the results of which showed leptin to be significantly associated with BMI (r=0.474, p<0.001). In addition, we found leptin to be positively correlated with both heart rate (r=0.061, p=0.049) and blood pressure (diastolic: r=0.106, p=0.001; systolic: r=0.076, p=0.015). We also found BMI to be significantly correlated with WHR (r=0.498, p<0.001).

We did not observe significant differences between the different alleles of the SNP rs16147 concerning levels of cortisol, ACTH, orexin and cotinin (data not shown).
Discussion

The novel finding of the present study is that the single nucleotide polymorphism rs16147, located in the NP-Y gene promoter, is significantly associated with both waist-hip ratio and serum leptin concentrations. These associations were sex specific, applying only to women. Our main result is not surprising, since various NP-Y gene variants have recently been found to be associated with obesity [30]. However, the evidence supporting the association between obesity and polymorphisms of the NP-Y gene has been inconsistent. In a large study, the functionally relevant NP-Y SNP rs16147 was not found to be associated with obesity, despite other SNPs covering the NP-Y gene having been found to be associated with obesity [30]. Our study confirmed this result and also found no association between BMI and rs16147. However, we did find rs16147 to be associated with the distribution of the body fat (central obesity). Furthermore, our finding is sex specific. Sexual dimorphism in human body composition is already well known and has been recently described: Compared to women, men are taller, they have lower overall fat mass, and their fat distribution, total lean mass, and bone mineral mass are also different [31]. Previous studies support our main finding, reporting that the genetic variance for WHR is significantly higher in women than in men [32]. The NP-Y gene thus accounts not only for BMI, as previous studies had suggested. Our results demonstrate that the NP-Y gene also accounts for body-fat distribution measured with WHR (an important obesity-related trait), and they also reveal a gene-by-sex interaction.

In a recently published paper by our group using the same data base, it could be demonstrated that smokers with a pathological eating behavior show an impaired neuroendocrine regulation of appetite and are prone to experience higher levels of stress and negative affectivity [26]. Altered psychological states (e.g. depression, stress) have been repeatedly associated with impaired neurobiological processes controlling food intake [11,33-35]. An important endocrinological mediator not only controlling for eating behavior, also acts as an important mediator in stress reactions and depressed mood, is leptin [36]. One finding of our study is that peripheral leptin levels are positively correlated with BMI, blood pressure, heart rate and waist-hip ratio, a finding which has already been reported repeatedly [37]. We found significantly higher peripheral leptin levels in women with the CT allele compared to female CC- and TT-allele carriers (rs16147). Previous studies had already found leptin levels to be higher in females than males if leptin levels are expressed as a percentage of body fat.[38,39]. Our result is surprising since most previous studies had found positive associations between leptin and BMI/WHR [40,41]. However, increased leptin levels have not always been found to
be associated with increased body weight [42,43]. As a restriction, it should be noted that in our study we assessed leptin levels cross-sectionally, and thus we could not discriminate between acute and chronic hyperleptinemia, although doing so is important for assessing leptin's long-term biological effects.

Taken together, our results suggest that the NP-Y system is involved in body-fat distribution in women. We found that individuals carrying the risk genotypes TT and CC of rs16147, which have been found to be associated with altered NP-Y levels in earlier studies [44-46], have both significantly lower peripheral leptin levels and a higher individual vulnerability to increased waist-hip ratio. These results were sex specific, implying additional risk factors contributing to the complex phenotype of obesity and waist-hip ratio. Our study does have some limitations: (a) The study findings are not generalizable to all people suffering from obesity as the primary goal of the study was not to study obesity but to investigate tobacco dependence in smokers and non-smokers. (b) We did not investigate whether rs16147 influences NP-Y and leptin expression directly, indirectly, or whether it acts through other pathways – a question that should be addressed in future studies. (c) Our study population lacked ethnic diversity, as the analysis was limited to individuals of German ancestry as a means of avoiding the effects of population stratification. For this reason, the study findings may not apply to populations of non-German descent. (d) There are many non-genetic factors that influence waist-hip ratio and leptin levels, including circadian rhythms, psychological factors, sex, addiction, as well as other hormones like insulin and cortisol, none of which were taken to account in the present study. However, the strengths of our analysis is, we found a so far unknown association in the NP-Y gene promoter that may constitute a genetic risk factor for elevated waist-hip ratio in woman. However, further studies are needed to replicate our preliminary findings.

To our knowledge, this is the first study showing a gene-by-sex interaction with an association between rs16147, leptin and waist-hip ratio. However, the pathogenesis of obesity and obesity-related-traits is complex and involves both genetic and environmental factors. Therefore, additional studies looking at different genes, exact (endo-)phenotypes and environmental factors would be useful contributions to this field.
References

Zondervan KT, Feitosa MF, Ferreira T, Allen HL, Weyant RJ, Wheeler E, Wood AR,
Estrada K, Goddard ME, Lettre G, Mangino M, Nyholt DR, Purcell S, Smith AV,
Visscher PM, Yang J, McCarroll SA, Nemesh J, Voight BF, Absher D, Amin N,
Aspelund T, Coin L, Glazer NL, Hayward C, Heard-Costa NL, Hottenga JJ, Johannsson
Lamina C, Leitzmann MF, McKnight B, Morris AP, Ong KK, Perry JR, Peters MJ,
Polasek O, Prokopenko I, Rayner NW, Ripatti S, Rivadeneira F, Robertson NR, Sanna
S, Sovio U, Surakka I, Teumer A, van Wijgden S, Vitart V, Zhao JH, Cavalcanti-
LJ, Silander K, Stark K, Tammeesoo ML, Teslovich TM, Timpson NJ, Watanabe RM,
Welch R, Chasman DI, Cooper MN, Jansson JO, Kettunen J, Lawrence RW, Pellikka
Bonnycastle LL, Bornstein SR, Buchanan TA, Campbell H, Day IN, Dei M, Dorr M,
Elliott P, Erds MR, Eriksson JG, Freimer NB, Fu M, Gaget S, Geus EJ, Gjesing AP,
Grellert H, Grassler J, Groves CJ, Guiducci C, Hartikainen AL, Hassanani N,
Havulinna AS, Herzig KH, Hicks AA, Hui J, Igl W, Jousilahti P, Jula A, Kajantie E,
Kinnunen L, Kolcic I, Koskinen S, Kovacs P, Kroemer HK, Krzelj V, Kuusisto J,
Kvaloy K, Laitinen J, Lantieri O, Lathrop GM, Luben RN, Ludwig B,
McArule WL, McCarthy A, Morken MA, Neils CA, Neils M, Neils AN, Neils G, Neils P,
Peden JF, Piikkinen KH, Platou CG, Pouta A, Ridderstrale M, Samani NJ,
Saramies J, Sinisalo J, Smit JH, Strawbridge RJ, Stringham HM, Swift AJ, Teder-
Laving M, Thomson B, Usala G, van Ommen GJ, Vatin V, Volpato CB,
Wallachofski H, Walters GB, Widen E, Wild SH, Willemen G, Witte DR,
Zgaga L, Zitting P, Beilby JY, James AL, Kahnonen M, Lehtimaki T, Nieminen MS,
Ohlsson C, Palmer LJ, Raitakari O, Ridker PM, Stumvoll M, Tonjes A, Viikari J,
Balkau B, Ben-Shlomo Y, Bergman RN, Boeing H, Smith GD, Ebrahim S, Fugroel P,
Hansen T, Hengstenberg C, Hveem K, Isomaa B, Jorgensen T, Karpe F, Khaw KT,
Laakso M, Lawlor DA, Marre M, Meitinger T, Metzalu P, Midtjell K, Pedersen O,
Salomaa V, Schwar P, Tuomi T, Tuomilehto J, Valle TT, Wareham NJ, Arnold
AM, Beckmann JS, Bergmann D, Boerwinkel E, Boomsma DI, Caulfield MJ, Collins
FS, Eiriksdottir G, Gudnason V, Gyllensten U, Hamsten A, Hattersley AT, Hofman A,
Hu FB, Illig T, Iribarren C, Jarvelin MR, Kao WH, Kaprio J, Launer LJ, Munro PB,
Oost A, Pennix BW, Pnamstaller PP, Psaty BM, Quertermous T, Rissanen A,
Rudan I, Shuldiner AR, Soroza N, Spector TD, Syvanen AC, Udai M, Uitterlinden A,
Volzke H, Vollenweider P, Wilson JW, Wittenman JC, Wright AF, Abecasis GR,
Boehnke M, Borecki IB, Deloukas P, Frayling TM, Groop LC, Haritunians T, Hunter
DJ, Kaplan RC, North KE, O’Connell JR, Peltonen L, Schlessinger D, Strachan DP,
Hirschhorn JN, Assimes TL, Wichmann HE, Thorsteinsdottir U, van Duijn CM,
Stefansson K, Cupples LA, Loos RJ, Barroso I, McCarthy MI, Fox CS, Mohlke KL,
Lindgren CM: Meta-analysis identifies 13 new loci associated with waist-hip ratio and
reveals sexual dimorphism in the genetic basis of fat distribution. Nat Genet;42:949-
960.

exacerbates depressive-like behavior in the Flinders Sensitive Line (FSL) rat, a genetic

34. Daubenmier J, Lin J, Blackburn E, Hecht FM, Kristeller J, Maninger N, Kuwata M,
Bacchetti P, Havel PJ, Epel E: Changes in stress, eating, and metabolic factors are
related to changes in telomerase activity in a randomized mindfulness intervention


