Sex-Specific Associations of SNVs rs324981 *NPSR1* and rs10914456 *HCRTR1* with Eating Disorders in Pakistani Adults: A Case-Control Study

Pasha Ghazal * and Kishwar Amin

Posted Date: 19 May 2026

doi: 10.20944/preprints202605.1185.v1

Keywords: hypocretin; *NPSR1*; eating disorders; sex difference; K-pop culture

 doi:10.20944/preprints202605.1185.v1

Article

Sex-Specific Associations of SNVs rs324981 *NPSR1* and rs10914456 *HCRTR1* with Eating Disorders in Pakistani Adults: A Case-Control Study

Pasha Ghazal * and Kishwar Amin

Department of Biosciences, COMSATS University Islamabad, Islamabad, Pakistan

* Correspondence: pasha.ghazal@comsats.edu.pk

Simple Summary

Eating disorders (EDs) represent an emerging yet underrecognized public health concern in Pakistan. Despite growing evidence, the actual prevalence is likely underestimated due to persistent mental health stigma and limited public awareness. Eating disorders are strongly intertwined with body-shape dissatisfaction, which is intensified by pervasive media portrayals of thinness, particularly among young women in Pakistan While sociocultural and psychological influences play a role, there is robust evidence of a strong genetic contribution. Neuropeptides are key regulators of appetite and reward, and are implicated in the pathophysiology of eating disorders. This study explored, the prevalent psycho-social risk factors and how genetic variation in Hypocretin and Neuropeptide S systems affects eating pathology in both males and females , who presented themselves at nutrition clinics . Results showed that females had more severe eating-related symptoms, especially restrictive eating and binge eating behaviors, and greater sensitivity to social influences including self-comparison with K-Pop celebrities. Although body dissatisfaction was equally common in both sexes. Importantly, we identified clear sex-specific genetic effects at two key variants: *HCRTR*1 rs10914456 and *NPSR1* rs324981. In females, the TT genotype of *HCRTR1* rs10914456 conferred a significantly higher risk, following a recessive pattern (risk increased when two copies were present). In contrast, in males, the T- allele of *NPSR*1 rs324981 showed a dominant effect, where even one copy increased susceptibility. These findings are novel, particularly in the context of Pakistan and South Asia, where such gene–environment interactions varied by sex in eating disorders have not been previously reported.

Abstract

Disordered eating in young adults is shaped by sociocultural pressures and may be modulated by genetic variation. We examined sex differences in eating-pathology, psychosocial correlates, at two candidate loci Hypocretin and Neuropeptide S (*HCRTR*1 rs10914456; *NPSR1* rs324981). A total of 550 individuals visiting various nutrition clinics were initially approached for participation in the study. Of these, 460 consented to take part ,after exclusions, 360 completed SCOFF; 200 scoring >2 proceeded to EAT-26 and comprised the analytic sample (100 males, 100 females). Psychosocial factors (media influence, academic pressure, peer pressure, isolation/loneliness, and K-pop self-comparison) were assessed by a structured questionnaire. EAT-26 total and subscales were compared by sex (t-tests). Genotypes were contrasted by sex using χ^2 tests; allele frequencies were derived from genotype counts and ORs with CI were computed. Females showed higher EAT-26 total scores than males (29.7±1.9 vs 23.2±1.3; $t(198)=2.82$, $p<0.005$); 68% of females and 76% of males scored ≥20. Anorexia subscale scores were greater in females ($t(198)=3.713$, $p<0.0003$), as well as binge-eating scores ($t(198)=1.722$, $p<0.05$); bulimia indices did not differ by sex ($p>0.05$). Body dissatisfaction was common (87%) without sex difference ($p>0.05$).Significant sex associations were observed for media influence ($\chi^2=67.94$, $p<0.05$), academic pressure ($\chi^2=45.6$, $p<0.0001$), K-pop self-comparison ($\chi^2=112.12$, $p<0.0001$), peer pressure ($\chi^2=46.37$, $p<0.05$),and isolation/loneliness ($\chi^2=28.72$, $p<0.0001$).Genotyping data revealed marked sex-dependent associations at both loci. For *HCRTR1* rs10914456, female cases

 doi:10.20944/preprints202605.1185.v1

showed a significantly higher frequency of the risk (TT) genotype, conferring 4.86-fold greater odds of carrying T-allele relative to males (OR = 4.86, 95% CI: 1.46–16.17, p = 0.001). In contrast, for *NPSR1* rs324981, males exhibited a pronounced T-allele–driven risk pattern, being T-carriers (AT+TT) relative to females (OR = 4.11, 95% CI 1.23–13.68, p = 0.022).Within females specifically, the AA genotype was significantly overrepresented compared with T-carrying genotypes (AA vs AT+TT: OR = 3.25, 95% CI: 1.59–6.66, p = 0.0013).Collectively, these results highlight a female-specific recessive risk pattern at *HCRTR1* and a male-specific dominant T-allele effect at *NPSR1*, underscoring robust sex-differentiated genetic susceptibility to disordered eating. Overall females exhibited severe eating-pathology and heightened psychosocial sensitivity than males, while genetic risk showed locus-specific sex patterns. Integrating psychosocial screening with genetic profiling may lead to early intervention.

Keywords: hypocretin; *NPSR1*; eating disorders; sex difference; K-pop culture

1. Introduction

Eating disorders (EDs) are serious and complex psychiatric conditions that significantly impair both psychological well-being and physical health [1]. An eating disorder can be described as persistent disturbance in eating behaviours or weight-control practices that lead to notable harm in physical or psychosocial functioning, and cannot be fully explained by other medical or psychiatric conditions [2]. The Diagnostic and Statistical Manual of Mental Disorders (DSM V) [3]classifies the major types of EDs as anorexia nervosa (AN) marked by extreme weight loss and distorted body image; bulimia nervosa (BN) and binge-eating disorder (BED) both characterized by episodes of consuming unusually large amounts of food accompanied by a sense of loss of control. In BN, these episodes are followed by compensatory behaviours like self-induced vomiting or misuse of laxatives and diuretics.

These disorders typically manifest between the ages of 15 and 25 years and often follow a chronic and relapsing course, with an average illness duration of about six years [4]. Alarmingly, a recent study found an average delay of more than five years between the onset of symptoms and seeking treatment [5]. Anorexia and bulimia disproportionately affect young women, while binge-eating disorder shows a more balanced sex distribution [6].

The prevalence of disordered eating is rising globally, frequently co-occurring with obesity. This is paralleled by younger age of onset and increasing healthcare requirements. Current estimates suggest that one in every six to seven young women is affected, with anorexia nervosa being as prevalent in adolescence as type-1- diabetes [7]. Moreover, mortality rates among individuals with EDs are nearly twice as high as in the general population and up to six times higher in those with anorexia nervosa. Moreover, up to 50% of individuals with BN or BED are currently obese or may become so, further elevating the risk for serious health complications [7].

In Pakistan, eating disorders (EDs) notably anorexia nervosa (AN), bulimia nervosa (BN), and binge eating disorders (BED) are increasingly affecting adolescents and young adults, particularly females [8]. However, there are fewer studies reporting the prevalence of eating disorders from Pakistan.

Eating disorders (EDs) represent an emerging yet underrecognized public health concern in Pakistan. Despite growing evidence, the actual prevalence is likely underestimated due to persistent mental health stigma and limited public awareness. Adolescent and young adult females (15–24 years) appear particularly at risk [9]. For instance, a recent study reported that 21% of female medical students in Lahore exhibited problematic eating behaviors [10]. While EDs are traditionally more common among women, rising body image dissatisfaction among men signals a broadening vulnerability.

Eating disorders are strongly intertwined with body-shape dissatisfaction, which is intensified by pervasive media portrayals of thinness, particularly among young women in Pakistan [11]. Urban

 doi:10.20944/preprints202605.1185.v1

populations, in particular, show higher rates of disordered eating, driven by the interplay of globalized beauty ideals, body dissatisfaction, peer and academic pressures, and underlying psychological distress such as anxiety and depression. For this study, participants were recruited from nutrition clinics, where they typically sought consultation for concerns related to diet, weight, or metabolic health. This setting facilitated in identification of individuals at higher risk of disordered eating behaviors.

In addition, we used a structured questionnaire to better understand the risk factors influencing such behaviors in these high risk groups such as the growing influence of K-pop culture, now highly popular among Pakistani youth; many adolescents aspire to emulate the extremely lean physiques of K-pop idols, which can heighten body dissatisfaction and encourage unhealthy weight-control practices [12]. Psychological and academic stress are well-documented risk factors for maladaptive eating in Pakistan. The country's highly competitive academic environment contributes to elevated levels of stress, anxiety, and depression, all of which are associated with disordered eating behaviors such as emotional restriction or binge eating used as coping strategies [13,14]. Peer pressure, deeply rooted in Pakistan's collectivist culture, represents an additional and powerful driver: social conformity expectations, appearance-related comparisons and interpersonal pressures during group interactions often push individuals ,especially those already insecure about their bodies, toward maladaptive eating patterns. Collectively, these psychosocial influences underscore the need for culturally informed screening tools and interventions that address the broader societal and psychological contributors to eating disorders in Pakistan.

While sociocultural and psychological influences play a role, there is robust evidence of a strong genetic contribution. Heritability estimates suggest that genetic factors account for approximately 56–84% of the risk for AN, 28–83% for BN, and 41–57% for BED [15,16].

Neurobiological and Genetic Basis for Targeting NPSR1 and HCRTR1 in Eating Disorders

Eating disorders arise from complex interactions between stress responsivity, affective regulation, and reward-driven feeding behaviors, processes that are strongly modulated by neuropeptide systems and show marked sex differences. Neuropeptides are key regulators of appetite, arousal, and reward, and dysregulation in their signaling is increasingly recognized in the pathophysiology of eating disorders [17,18]. Orexin (Hypocretin), an orexigenic neuropeptide produced in the lateral hypothalamus, promotes food-seeking behavior, particularly in response to palatable stimuli and stress [19,20]. Elevated orexin signaling has been linked to binge-eating tendencies and heightened reward sensitivity, features often observed in bulimia nervosa and binge eating disorder [21]. In contrast, neuropeptide S (*NPS*) exerts anorexigenic effect by promoting satiety and suppressing anxiety-like behavior [22,23], which may help modulate compulsive eating [24,25]. Variants affecting Hypocretin and *NPS* systems could therefore contribute to disordered eating by altering the balance between reward-driven hunger and satiety regulation. Based on this neurobiological framework, the present study focused on two functionally relevant neuropeptide receptor gene variants *NPSR1* rs324981 (Asn107Ile) and Hypocretin receptor 1 *(HCRTR1*) rs10914456 selected a priori due to their established roles in stress, arousal, and feeding-related neural circuits.

The *NPSR*1 rs324981 polymorphism is a well-characterized functional variant that alters receptor signaling efficacy and downstream stress responsivity. Human genetic and neuroimaging studies have consistently linked this variant to anxiety, panic disorder, and heightened amygdala reactivity, phenotypes closely associated with emotional dysregulation and stress-induced eating behaviors [26]. Previously we have reported association of T- allele in *NPSR1* rs324981 with obesity in male Pakistani population [27]. The Hypocretin system, acting primarily through *HCRTR1*, plays a central role in arousal regulation, reward processing, impulsivity, and feeding initiation. Preclinical studies show that orexin signaling promotes binge-like eating and compulsive reward seeking, with pronounced sex-dependent effects. The *HCRTR1* rs10914456 , on the other hand, has been implicated in emotional regulation, sleep–wake balance, and psychiatric conditions such as depression and anxiety [28].

 doi:10.20944/preprints202605.1185.v1

Functionally, rs324981 gene variant in *NPSR1* results in a missense change (Asn107Ile) that has been shown to alter receptor signaling efficiency, potentially influencing arousal, stress responsiveness, and feeding behavior. Although rs10914456 gene variant in *HCRTR1* is intronic, it may affect gene expression or splicing, thereby modulating orexinergic signaling. Together, these variants likely contribute to eating-related phenotypes through regulatory and receptor-level mechanisms rather than acting as outright causal mutations.

Despite strong experimental evidence, human genetic studies examining *HCRTR1* and *NPSR1* variation in eating disorders remain scarce.These two SNVs were therefore selected for their neurobiological relevance and prior evidence linking them to affective dysregulation, providing mechanistic basis to explore their potential role in eating disorders.

Based on this prior evidence, we hypothesized that functional variation in *NPSR1* and *HCRTR1* might confer sex-specific genetic susceptibility to eating disorder phenotypes, reflecting differential engagement of stress- and arousal-related neurocircuits in males and females. This study, aimed to systematically investigate the association of these variants with eating disorder risk, integrating genetic, psychosocial, and sex-stratified analyses.Therefore, we sought to1) quantify and compare sex differences for disordered-eating prevalence and severity .2) To evaluate how media exposure, academic pressure, peer influence including appearance comparison with K-pop idols, isolation/loneliness, and harassment relate to disordered-eating outcomes, and to test whether these associations differ between males and females.3)To investigate sex-specific genetic variations in the *HCRTR1* SNV rs10914456 and *NPSR1* SNV rs324981, and their association with eating-related phenotypes by comparing genotype and allele frequencies between males and females recruited from nutrition clinics, as well as examining how these genotypes and alleles relate to EAT-26 total scores.

2. Methodology

2.1.1. Study Design

This is a case-control, multi-center study exploring association of single nucleotide gene variants (SNVs) in receptor genes of *HCRTR1* rs10914456 and *NPSR1 rs324981* with eating disorders among Pakistani young adults, who were recruited from nutrition clinics.

2.1.2. Study Area

The recruitment for study participants was made over the course of six months starting from March 2024 till August 2024 by visiting nutrition and obesity clinics in various public -private hospitals situated in Islamabad, Rawalpindi and Faisalabad

2.1.3. Study Population

A total of 550 individuals visiting various nutrition clinics were initially approached for participation in the study. Of these, 460 consented to take part. Based on pre-defined exclusion criteria, 100 participants were excluded. SCOFF questionnaire was administered to the remaining 360 participants for initial screening of eating disorder symptoms .Participants who scored ≥2 on the SCOFF (n = 200) were invited to complete the EAT-26 questionnaire for further assessment. Based on Eat-26 scores, 76 females and 68 males were classified as likely having an eating disorder, while 24 females and 32 males were categorized as controls. All these individuals willingly consented to be part of genotype screening for the *HCRTR1* rs1094456 and *NPSR1* rs324981 SNVs.

2.1.4. Sampling and Data Collection

Patients visiting nutrition clinic were recruited based on clinicians initial screening for the inclusion/ exclusion criteria. Patient recruitment occurred only after a doctor/nutritionist was assigned by the nutrition department to assist with the research. The patient's history was then assessed, with the assigned doctor present, to ensure they met the inclusion criteria. Patients were

 doi:10.20944/preprints202605.1185.v1

informed of the study objectives, and consent was obtained in the presence of their attendee. After consent, privacy was requested to avoid any external influence on the patient's responses.

2.2.1. Inclusion Criteria

All those subjects who were within the age range 18-60 years and were mentally stable and were visiting nutrition clinics were approached for the interview.

2.2.2. Exclusion Criteria

Subjects diagnosed with severe psychiatric comorbidities, significant medical illnesses, medications affecting appetite or mood, recent participation in other studies, pregnancy, and cognitive impairment, diagnosis of type 1 or type 2 diabetes were excluded, as well as those with chronic conditions such as Alzheimer's, heart disease, or prior psychological disorders (e.g., schizophrenia, schizoaffective disorder, seasonal affective disorder, cyclothymia, substance-induced mood disorder, minor depression, or adjustment disorder with depressed mood).

2.3. Study Approval and Ethics

The study adhered to the Helsinki Declaration of 1975 (revised in 2013) and all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and was approved by the Ethics Review Board of Comsats University Islamabad (CUI/BIO/ERB/2024/41). Participants were briefed on the study's objectives and were free to withdraw at any point during data and sample collection. Written informed consent was obtained, and confidentiality and anonymity of patient data were maintained throughout the study

2.3.1. Study Questionnaire

The study questionnaire had four parts. The first part collected demographic data such as age, income, education, locality, occupation and BMI .Patients were interviewed in Urdu if they had difficulty with English. The second part employed a self- structured questionnaire to evaluate , cultural , social and psychological risk factors influencing eating attitudes among Pakistani adults. Whereas, the third and fourth parts of the questionnaire comprised of SCOFF and EAT -26 tests respectively.

2.3.2. SCOFF Test

The SCOFF questionnaire is a brief, five-item screening tool designed to identify individuals at risk for eating disorders, particularly anorexia nervosa and bulimia nervosa. Each item corresponds to a key feature of these disorders, forming the acronym SCOFF: Sick (inducing vomiting), Control (loss of control over eating), One stone (significant weight loss), Fat (perception of being fat), and Food (preoccupation with food). Participants respond to each question with a "Yes" or "No" answer. Each "Yes" response scores one point, and a total score of two or more suggests a likely case of an eating disorder, warranting further clinical evaluation. The SCOFF questionnaire has demonstrated high sensitivity (100%) and specificity (87.5%) in various populations, making it an effective initial screening instrument in both clinical and non-clinical settings [29].

2.3.3. EAT-26 (Eating Attitudes Test—26)

The Eating Attitudes Test-26 (EAT-26) is a widely used standardized tool for identifying disordered eating attitudes and behaviors. It comprises of 26 items that assess domains such as body dissatisfaction, dieting practices, bulimic tendencies, and food-related pre-occupations. Participants respond to each item using a 6-point Likert scale (Never, Rarely, Sometimes, Often, Usually, Always). The responses are scored and summed to yield a total score, with higher scores indicating greater concern related to eating behaviors. A total score of 20 or above is generally considered indicative of a potential eating disorder risk such as anorexia nervosa, bulimia nervosa, and binge eating disorder [30].

 doi:10.20944/preprints202605.1185.v1

2.3.4. SCOFF and EAT-26 Administration to the Study Population

The SCOFF questionnaire was initially administered as a screening tool to identify individuals at risk for eating disorders. Participants who scored ≥2 on the SCOFF (n = 200) were subsequently invited to complete the Eating Attitudes Test-26 (EAT-26) for further assessment of disordered eating behaviors.

Based on EAT-26 scores, participants scoring ≥ 20 were classified as likely having an eating disorder, while those scoring < 20 were categorized as controls. Using this criterion, 76 females and 68 males were classified as probable eating disorder cases, whereas 24 females and 32 males were categorized as controls.

2.4. Blood Sample Collection Details

We ascertained relatively homogeneous genetic background of our study population in order, to preclude factor of population stratification affecting our study results.

Blood samples were collected by vein puncture in 5 ml tubes containing Ethylenediamine tetra acetic acid (EDTA), from a total of 200 recruited participants .The tubes were centrifuged for plasma and cell separation. Total DNA was extracted using the salting out method described by [31].

2.4.1. Primer Designing

Specific forward and reverse primers pairs of tetra-ARMS design were designed for the selected targeted gene SNV using Primer 3 version 0.4.0 software (http://bioinfo.ut.ee/primer3-0.4.0/primer3/).

2.4.2. Genetic Analysis (HCRTR1 rs10914456)

Genotyping of *HCRTR1* rs10914456 polymorphism was performed using the following pairs of tetra-ARMS PCR primers designed by our lab: Forward Outer: AGCCCTGTAGTCCACCAACACTCATCAT, Reverse Outer:

GGACACTCAAATCACCCACCACAGAG, Forward inner:

CAGGCACGATCCTCCTCATTTGACATAT, Reverse inner:

TCATTTTCCTTTCTAAGCCTTGCTTTCCTG

Briefly, genomic DNA was amplified for *HCRTR1* rs10914456 in a 25 µl total reaction volume using a set of four primers in one reaction. In addition, this reaction included 100 mmol/l of dNTP, 3mmol/l of MgSO4, 1X PCR amplification buffer (20mmol/l Tris–HCl pH 8.4, 50 mmol/l KCl), 1X PCRx Enhancer cosolvent (Invitrogen, Carlsbad, California, USA) and 1U thermofischer Taq DNA polymerase (Novagen, Gibbstown, New Jersey, USA). Amplification of the gene was done using thermal cycler (Thermo Electron Corporation) using a set of 4 primers using following conditions: 95 °C for 5 mins, followed by 35 cycles of 95 °C for 30 secs (denaturation), 58 °C 30 secs (annealing), 72 °C for 45 secs (extension), and final extension for 4 mins at 72 °C .The primers amplified two allele-specific amplicons (161bp and 119 bp) and the entire region (222 bp) as an internal control, which were resolved on 2% agarose gel. Gel electrophoresis was carried out for 45 min at 95 V and 400 mA followed by visualization under UV light using the trans-illuminator.

2.4.3. Genetic Analysis (NPSR1 rs324981)

Genotyping of the *NPSR1* rs324981 gene variant was performed using the tetra-ARMS PCR primers designed by our lab. Outer Forward *NPSR1*-OF: 5′-TCCCCAGGCATTTCTATT-3′ 345 *NPSR1*-OuterReverse: 5′-CTCTGGTATTTGTCTCATCA-3 *NPSR 1*-Inner Forward: 5′-GTCAACAGACAGATATGAA-3′ *NPSR 1*-Inner Reverse: 5′-TCTCCAGTCGCCCAC-

Briefly, genomic DNA was amplified for *NPSR1* rs324981 in a 25 µl total reaction volume using a set of four primers in one reaction. In addition, this reaction included 100 mmol/l of dNTP, 3mmol/l of MgSO4, 1X PCR amplification buffer (20mmol/l Tris–HCl pH 8.4, 50 mmol/l KCl), 1X PCRx Enhancer cosolvent (Invitrogen, Carlsbad, California, USA) and 1U thermofischer Taq DNA polymerase (Novagen, Gibbstown, New Jersey, USA). Amplification of the gene was done using

 doi:10.20944/preprints202605.1185.v1

thermal cycler (Thermo Electron Corporation) using a set of 4 primers using following conditions: 95 °C for 5 mins, followed by 35 cycles of 95 °C for 30 secs (denaturation), 52 °C 30 secs (annealing), 72 °C for 45 secs (extension), and final extension for 4 mins at 72 °C· The primers amplified two allele-specific amplicons (155 and 232 bp) and the entire region (345 bp) as an internal control, which were resolved on 2% agarose gel.Gel electrophoresis was carried out for 45 min at 95 V and 400 mA followed by visualization under UV light using the trans-illuminator.

2.4.4. Genotyping Success and Quality Control

Genotyping was successfully completed for all analyzed samples, achieving an overall genotyping success rate of 99% for both *HCRTR1* rs10914456 and *NPSR1* rs324981 loci. To verify reliability, approximately 10% of the samples were re-genotyped in duplicate, yielding a replicate concordance rate of ≥98%, confirming high analytical precision. Samples with ambiguous or missing genotype calls (<1%) were excluded from further analysis to maintain data integrity.

2.4.5. SCOFF and Eat-26 Reliability and Construct Validity for the Study Population

In our study population, we assessed the construct reliability of Eat-26 and SCOFF test using Cronbach's alpha. Overall, for our study population, Eat-26 had an alpha (α) value of 0.83 for 26 items which showed good reliability. Concerning validity, in Pearson's correlation test, all study questions were significantly correlated at 0.01 level (two-tailed).Similarly, SCOFF had an alpha (α) value of 0.80 for 5 items which showed good reliability. Concerning validity, in Pearson's correlation test, all study questions were significantly correlated at 0.01 level (two-tailed).

2.5. CFA Analysis

For the EAT-26, Confirmatory Factor Analysis (CFA) was conducted using the original 3-factor model (Dieting, Bulimia & Food Preoccupation, Oral Control) to examine the underlying factor structure of disordered eating. The CFA showed moderate fit indices, suggesting a reasonable representation of the data with an overall acceptable fit to the data ($\chi^2(296) = 639.38$, $p > 0.001$; CFI = 0.91; NFI = 0.92). The strong standardized factor loadings for the Dieting subscale, such as Q6 (0.90), Q11 (0.91), and Q23 (1.07), indicate reliable measurement of dieting behaviors. Similarly, the Bulimia & Food Preoccupation factor demonstrated strong loadings for Q3 (0.79), Q4 (0.65), and Q18 (1.03).Lastly, the Oral Control subscale also exhibited reliable loadings for items like Q2 (0.82) and Q5 (0.85).

2.6. Statistical Analysis

Quantitative values were expressed as mean, frequency, percentage, and standard deviation. Group differences were analyzed using Student's t-test, and associations were determined with the Chi-square test. To control for potential inflation of Type I error due to multiple comparisons, p-values from chi-square analyses were adjusted using the Benjamini–Hochberg false discovery rate (FDR) procedure, with statistical significance defined as **$q < 0.05$**.

Odds ratios with 95% confidence intervals were calculated to estimate relative risks. Direct counting method was used to obtain the genotype frequencies of screened SNVs. Hardy–Weinberg equilibrium was tested via Chi-square ($\chi 2$) goodness of fit test to compare the observed and expected genotype frequencies among controls. Statistical analysis to compare distributions of allele and genotype frequencies between cases and controls were performed using Chi-square ($\chi 2$) test as appropriate. The major allele identified in the control group was used as the reference category for all genetic association analyses, with the **C allele (CC genotype)** for *HCRTR1 rs10914456* and the **A allele (AA genotype)** for *NPSR rs324981* serving as baseline genotypes against which alternative alleles and genotypes were compared. Mixed-effects two-way ANOVA was applied to assess the independent and interactive effects of sex and genotype on phenotypic measures. When overall model effects were detected, Holm–Sidak post-hoc comparisons were conducted to identify specific

 doi:10.20944/preprints202605.1185.v1

pairwise differences. Genotype data were analyzed using **allele-dose logistic regression**, coding genotypes as 0, 1, or 2 copies of the minor allele (T). Analyses were **sex-stratified** to account for observed sex-specific effects. For *HCRTR1* rs10914456, the T allele was treated as the minor allele, and for *NPSR1* rs324981, the T allele was the minor allele. **Covariates included age, body mass index (BMI), and gender (where appropriate) and Eat-26 scores**. Logistic regression models estimated the **odds ratio (OR) per additional minor allele**, along with 95% confidence intervals (CIs) and p-values. To complement allele-dose models, genotype-level comparisons under **dominant and recessive models** were also performed to evaluate non-linear effects and confirm sex-specific associations. Data analysis was performed using SPSS (v.23) **Python (stats models) and** GraphPad Prism 9, with significance set at $p<0.05$.

3. Results

3.1. Demographic Trends of the Study Participants

Overall ,study participants(n=200) had a mean age of 28.65 ± 0.95 years, with no significant difference between cases (28.27 ± 1.13) and controls (29.50 ± 1.18) ($t = 2.16$, $p > 0.05$). Similarly, the distribution of age groups, education level, income, locality, and occupation did not differ significantly between cases and controls (all $p > 0.05$). Although mean BMI values were similar between cases and controls, the distribution of BMI categories differed significantly ($\chi^2 = 13.1$, $p < 0.01$), with a higher proportion of cases in the pre-obese category than controls (see Table 1).

Table 1. Demographic and clinical characteristics of Study population , N=200, p<0.05.

Characteristics	Total (n=200)	Controls (n=56)	Cases (n=144)	χ^2 / t	P-value
Mean age (years)	28.65 ±0.95	29.50 ±1.18	28.27±1.13	2.16	> 0.05
Age groups					
16–35	148 (74%)	40 (71%)	108 (75%)	0.27	> 0.05
35–55	52 (26%)	16 (29%)	36 (25%)		
Education					
≤12 years	102 (51%)	28 (50%)	74 (51%)	0.03	> 0.05
>12 years	98 (49%)	28 (50%)	70 (49%)		
Income					
<50k	60 (30%)	20 (36%)	40 (28%)	1.2	> 0.05
50–200k	140 (70%)	36 (64%)	104 (72%)		
Locality					
Urban	138 (71%)	36 (64%)	102 (71%)	0.8	> 0.05
Rural	62 (29%)	20 (36%)	42 (29%)		
Occupation					
Employed	124 (62%)	38 (68%)	86 (60%)	1.3	> 0.05
Unemployed	78 (39%)	18 (32%)	60 (40%)		
BMI (Mean)	26.39 ±0.62	26.16 ±1.25	26.93 ±1.15		
BMI categories					
Underweight (<18.5)	24 (12%)	12 (21%)	12 (8%)	13.1	< 0.01
Normal (18.5–24.9)	58 (29%)	16 (29%)	44 (31%)		
Pre-obese (25–29.9)	54 (27%)	7 (13%)	48 (33%)		
Obese class I (30–34.9)	52 (26%)	18 (32%)	31 (22%)		
Obese class II (35–40)	12 (6%)	4 (7%)	8 (6%)		

3.2. Demographic Trends Varied by Sex

We, next explored demographic characteristics differed by sex, revealing several notable patterns.

The panel in this study originally consisted of 200 participants, of which 100 were males and were classified in to (controls=24, cases =76) based upon Eat-26 scores, similarly, study population

 doi:10.20944/preprints202605.1185.v1

comprised of an equal number of females (controls=32, cases =68). All the participants were recruited from nutrition clinics. The mean age of the participants was 28.6± 0.95 years and the mean BMI was 26.39 ± 0.62 kg/m2. Overall large majority (74%) of the participants were in the middle age (26-35 years) bracket, (χ2=4.16, p>0.05). Education level differed markedly between groups (χ^2 = 13.84, p < 0.001), with a higher proportion of males and cases having education above 12 years. There was no disparity among groups income wise(χ^2 = 4.96, p > 0.05)

Majority of participants were city dwellers (71%) whereas considerable participation was made from the rural areas as well (29%)(see Table 1). With regard to BMI, significantly, greater number of women participants among cases fell in the obese class I(29%) and class II (8%) in contrast to male cases (χ2 = 55.7, p<0.0001). Employment status also showed a highly significant difference (χ^2 = 57.8, p<0.0001), with a majority of female cases being unemployed, whereas most male participants (both cases and controls) were employed (see Table 1). Overall, these findings indicate that education, occupation, and BMI were the most influential socio-demographic correlates distinguishing case and control groups, highlighting potential sex-specific vulnerability patterns (see Table 2)

Table 2. Socio-demographic characteristics and BMI of the study population(N=100). Chi-square analysis of differences between males and females , p<0.05.

Characteristics	Total(n=200)	Control Females (n=32)	Cases Females (n=68)	Controls Males (n=24)	Cases males (n=76)	Chi- square statistics χ2	*P*-values statistics
Mean age	28.65 ±0.952	29.81 ±2.34	30.52 ±1.75$	28.92 ±2.66	30.32± 1.43$	4.16	> 0.05
Age groups							
16-35	148(74)	22(69)	46(68)	18(75)	62(82)		
35-55	52(26)	10(31)	22(32)	6(25)	14(18)		
Education							
≤12years	102(51)	20(63)	44(65)	8(33)	30(39)	13.843	< 0.001
>12years	98(49)	12(37)	24(35.29)	16(66.6)	46(60.5)		
Income							
<50k	60(30)	14(44)	16(24)	6(25)	24(32)	4.96	> 0.05
50-200k	140(70)	18(56)	52(76)	18(75)	46(68)		
Locality							
Urban	138(71)	16(50)	46(68)	20(83)	56(74)	8.54	< 0.05
Rural	62(29)	16(50)	22(32)	4(16)	20(26)		
Occupation							
Employed	124(62)	10(41)	8(12)	28(88)	50(66)	**57.8**	**< 0.0001**
Unemployed	78(39)	14(58)	60(88)	4(13)	26(34)		
BMI	26.39±0.62	24.5±2.52	27±1.24	23.50±0.75	26.86±1.06		
<18.5 (Underweight)	24(12)	2(6)	10(13)	10(41)	2(3)		
18.5-24.9 (Normal)	58(29)	12(38)	24(32)	4(17)	20(29)		
25-29.9 (Pre-Obese)	54(27)	4(13)	14(18)	2(8)	35(50)	**55.7**	**< 0.0001**
30 to 34.9 (Obese class 1)	52(26)	12(38)	22(29)	6(25)	9(14)		
35to40 (Obese class2)	12(6)	2(6)	6(8)	2(8)	2(3)		

 doi:10.20944/preprints202605.1185.v1

3.3. Cultural, Social and Psychological Risk Factors Influencing Eating Attitudes

The findings revealed several notable genders-specific patterns in eating behavior, with multiple factors showing statistically significant associations. Overall, a substantial proportion of participants (87%) reported dissatisfaction with their body image, but no gender difference emerged ($\chi^2 = 0.54$, $p > 0.05$) and 86% of both genders exhibited non- satisfactory self- perception of body. In contrast, media influence on self-image was strongly associated with gender ($\chi^2 = 67.94$, $p < 0.0001$, $q=0.0006$); the majority of females (42%) viewed media influencing negatively , whereas males (82%) predominantly perceived it as neutral or positive. Academic stress also played a significant role ($\chi^2 = 45.6$, $p < 0.0001$,$q=0.0003$), with a higher percentage of females (36%) engaging in stress-induced binge eating compared to 18% of males. While a greater majority of males (70%) reported no impact of academic pressure on their eating behavior. Gender differences were most pronounced in appearance-related self-comparison with Korean-popular artists ($\chi^2 = 112.12$, $p < 0.0001$, 0.0002), where 80% of females showed dissatisfaction with their body image in comparison to K-pop artists, in contrast only 12% males exhibited similar behavior. The effect of peer- pressure on eating behavior was similarly gendered ($\chi^2 = 46.37$, $p < 0.0001$, $q=0.00015$), impacting 78% of females compared to 22% of males. Additionally, responses to isolation or loneliness showed significant gender disparities ($\chi^2 = 28.72$, $p < 0.0001$, 0.00012); emotional binge (44%) and stress eating (22%) were notably more frequent among females (66%).Whereas, a good majority of males (26%) reported "seeking of social support" to combat impact of isolation ,however, this behavior was reported only by 4% of females. All together, these results highlight that females are disproportionately affected by psychosocial and socio-cultural factors shaping disordered eating behaviors(see Table 3).

Table 3. Self-awareness questionnaire on the eating disorders, N=100 . Chi-square analysis of the variables between males and females, p<0.05.All p values were adjusted for Benjamin-Hochberg false discovery rates.

Question	Total (N=200)	Males (N=100)	Females (N=100)	Chi-square statistics	*P- value*	*FDR q values*
Self-Perception of body image ?						
Satisfied	26 (13%)	(14)	(14)	0.54	> 0.05	> 0.05
Dissatisfied	174 (87%)	(86)	(86)			
Effect of media on one's self-image?						
Positive impact	44 (22%)	(10)	(34)	67.94	**< 0.0001**	**0.0006**
Negative impact	50 (25%)	(8)	(42)			
Neutral	106 (53%)	(82)	(24)			
Impact of Academic pressure on eating?						
Stress-induced Binge Eating	56 (28%)	(18)	(36)	45.6	**< 0.0001**	**0.0003**
Positive Impact	10 (5%)	(6)	(2)			
Neutral	94 (47%)	(70)	(28)			
Stress induced Diet	40 (20%)	(6)	(34)			
Self-comparison with appearance of K-pop idols						

 doi:10.20944/preprints202605.1185.v1

Satisfied	98 (49%)	(88)	(10)	112.12	**< 0.0001**	**0.0002**
Dissatisfied	102 (51%)	(12)	(80)			
Effect of peer pressure on one's eating behavior?						
Affected	106 (53%)	(30)	(78)	46.37	**< 0.0001**	**0.00015**
Not affected	94 (47%)	(70)	(22)			
Impact of isolation/loneliness on eating habits?						
Emotional binge eating	70 (35%)	(26)	(44)	28.72	**< 0.0001**	**0.00012**
Seeking social support	30 (15%)	(26)	(4)			
Stress eating	30 (15%)	(8)	(22)			
Neutral	70 (35%)	(40)	(30)			

3.4. Eat-26 Scores of Study Population

Analysis of EAT-26 scores revealed significant gender differences in disordered eating attitudes and behaviors. The overall average EAT-26 score was significantly higher among females (29.7 ± 1.9) in comparison to males (23.2 ± 1.3), indicating a greater tendency toward eating concerns among women ($t(198) = 2.82$, $p < 0.005$).Overall, 72% of the total study population scored above the clinical cutoff (≥ 20), with females (68%) scoring significantly higher (35.86±1.8) than males(76%) (28.05±1.108) ($t(198)=3.713$, $p<0.0003$).These observations indicated heightened vulnerability among females towards disordered eating. Scores on the anorexia sub-scale were also significantly elevated among females (15.35± 1.361) versus males (12.6± 0.864), reflecting a gender disparity in restrictive eating behaviors ($t(198) = 2.166$, $p < 0.05$). Similarly, the binge eating sub-scale showed higher scores in females(7.4±0.782) than males (5.4±0.621), a difference that reached statistical significance ($t(198)=1.72$, $p < 0.05$). Although females had slightly higher bulimia scores (5.16 ±0.558) than males (4.48 ±0.535), this difference was not statistically significant ($t(198)=0.87$, $p> 0.05$). Additionally, no significant gender difference was found in scores on the bulimia cut-off subscale ($t(198) = 0.87$, $p > 0.05$).These findings underscore the increased risk of eating pathology in females, especially concerning anorexic and binge-eating tendencies.(see Table 4)

Table 4. Eat-26 test scores of the study population(N=200). P<0.05.

Eat-26 scores	**Total (n=200)**	**Male N=100**	**Female N=100**	**Statistics**	**P -value**
Mean ± SD	26.74±1.226	23.2± 1.3	29.7± 1.9	t(198)=2.82	< 0.005
Participants with Cutoff score= <20 (n%)	56(28)	(24)	(32)		
Mean score± SD	13.10±0.959	13.37±1.214	12.75±1.605	t(198)=0.30	> 0.05
Participants with Cutoff score= ≥20	144(72)	(76)	(68)		

 doi:10.20944/preprints202605.1185.v1

Mean score ± SD	32.18±1.168	28.05±1.108	35.86±1.8	t(198)=3.713	< 0.0003
Anorexia (n%)	46(23)	(14)	(32)		
Mean ± SD	13.97±0.814	12.6± 0.864	15.35± 1.361	t(198)=2.166	< 0.05
Binge eating(n%)	10(5)	(2)	(8)		
Mean ± SD	6.54 ±0.504	5.4±0.621	7.4±0.782	t(198)=1.722	< 0.05
Bulimia Nervosa (n%)	10(5)	(2)	(8)		
Mean ± SD	4.82 ±0.386	4.48 ±0.535	5.16 ±0.558	t(198)=0.87	> 0.05

3.5. Correlation of Genotypic/Allele Frequencies of HCRTR1 rs10914456 and NPSR1 rs324981 with Eating Disorders

Hardy–Weinberg equilibrium (HWE) was assessed separately for male and female control groups to verify genotypic distribution stability. For **males**, both SNVs conformed to equilibrium expectations, with ***HCRTR1* rs10914456** showing ($\chi^2 = 0.26$, $p = 0.61$), and ***NPSR1* rs324981** showing ($\chi^2 = 0.09$, $p = 0.76$), indicating no significant deviation from HWE. Similarly, in **female controls**, genotype distributions were consistent with HWE for both ***HCRTR1* rs10914456** ($\chi^2 = 0.31$, $p = 0.57$) and ***NPSR1* rs324981** ($\chi^2 = 0.12$, $p = 0.73$). These findings suggest that genotyping was reliable and that allele and genotype frequencies in the control groups were representative of the studied population, minimizing the likelihood of selection bias or genotyping error. Sex-based comparisons revealed striking differences in the distribution of both risk and protective alleles for *HCRTR1* rs10914456 and *NPSR1* rs324981. For *HCRTR1* rs10914456, the TT genotype is considered the risk variant, and females exhibited a substantially higher frequency of the TT risk genotype (23.5%) compared to males (5.3%), while males showed predominance of the heterozygous TC genotype (50%).The difference across genotypes was highly significant ($\chi^2 = 10.53$, $p < 0.001$). Interestingly, the protective CC genotype was observed more frequently among males (44.7%) than among females (41.2%).Allelic trends supported this pattern, with the T risk allele more common in females (41.2%) than males (30.3%),while the protective C allele was enriched in males(69.7%) indicating a stronger *HCRTR1*-linked vulnerability among females associated with this locus ($p < 0.05$) (see Table 5)

Table 5. The distribution of genotype and allelic frequencies of *HCRTR1* rs10914456 and *NPSR1* rs 324981 gene variants between males and females cases identified via Eat-26 test. p<0.05.

Genotype / Allele	Female Cases (N = 68) n (%)	Male Cases (N = 76) n (%)	χ^2	p-value
***HCRTR1* rs10914456**				
CC	28 (41.2%)	34 (44.7%)		
TC	24 (35.3%)	38 (50.0%)	10.53	**< 0.001**
TT	16 (23.5%)	4 (5.3%)		
C allele frequency	0.588 (58.8%)	0.697 (69.7%)	3.75	< 0.05
T allele frequency	0.412 (41.2%)	0.303 (30.3%)		
***NPSR1* rs324981**				
AA	52 (76.5%)	38(50%)	10.92	**< 0.001**

 doi:10.20944/preprints202605.1185.v1

AT	12 (17.6%)	26 (34.2%)		
TT	4 (5.9%)	12 (15.8%)	12.93	< 0.001
A allele frequency	0.853 (85.3%)	0.671 (67.1 %)		
T allele frequency	0.147 (14.7%)	0.329 (32.9%)		

For *NPSR1* rs324981, an opposite pattern was observed. The AA genotype was markedly more prevalent in females (76.5%) than males (50%), whereas males showed higher frequencies of the AT and TT risk genotypes (34.2% and 15.8%, respectively).The difference across genotypes was highly significant ($\chi^2 = 10.92$, $p < 0.001$).Allele frequency analysis further reinforced this pattern: the T allele, associated with increased risk, was more than twice as frequent among males (32.9%) as compared to females (14.7%), while the protective A- allele was significantly enriched among females (85.3%) ($\chi^2 = 12.93$, $p < 0.001$).

These findings indicated that although females showed higher risk genotype frequency for *HCRTR1* rs10914456, females overall exhibited an increased burden of genetic risk across both loci, particularly for *HCRTR1* rs10914456, where the protective allele was substantially less frequent. Overall, *NPSR*-linked genetic susceptibility was more prominent in males, whereas *HCRTR1* rs10914456 -associated risk was more female-driven.

3.6. Sex-Dependent Genotypic Associations of HCRTR1 and NPSR1 Variants with Eating Disorder Risk

Genotype-level analyses revealed opposing, sex-specific patterns at the two loci. For *HCRTR1* (rs10914456) the signal was driven by TT homozygosity in females: female cases had substantially higher odds of being TT than male cases (TT vs CC OR = 4.86, 95% CI 1.46–16.17, p = 0.01), and the recessive contrast was similarly strong (TT vs CC+TC: OR = 5.54, 95% CI 1.75–17.49, p = 0.003). By contrast, the heterozygote comparison and dominant model showed no significant sex difference (TC vs CC, male vs female: OR = 0.77, 95% CI 0.38–1.57, p = 0.47; dominant TC+TT vs CC: OR = 1.16, 95% CI 0.60–2.24, p = 0.67), indicating that the HCRTR1 effect is best characterized as a female-specific recessive signal.

For *NPSR1* rs324981 the pattern was found to be inverse: male cases carried TT-containing genotypes at much higher odds than male cases. Heterozygotes were nearly three times more likely in males (AT vs AA, OR = 2.96, 95% CI 1.33–6.61, p = 0.007), and TT homozygotes were also enriched (TT vs AA OR = 4.11, 95% CI 1.23–13.68, p = 0.022). Under a dominant model (AT+TT vs AA) males had markedly greater odds of carrying any T allele (OR = 3.25, 95% CI 1.59–6.66, p = 0.0013). The recessive TT comparison (male vs female) trended in the same direction but was underpowered (OR = 3.00, 95% CI 0.92–9.78, p = 0.069).

Whereas, among females specifically, the AA genotype was significantly overrepresented compared with T-carrying genotypes (AA vs AT+TT: OR = 3.25, 95% CI: 1.59–6.66, p = 0.0013), whereas in males, T allele carriage (AT/TT) dominated. In sum, genotype analyses indicate a female-specific recessive effect at *HCRTR1* rs10914456 (TT) and a robust male-predominant effect at *NPSR1* rs324981 (AT and TT; dominant model); these model-specific results are internally consistent with the ANOVA/post-hoc patterns and the study's power profile.

3.7. Sexually Dimorphic Effects of NPSR1 rs324981 and HCRTR1 rs10914456 Genotypes on Eat-26 Scores Among Study Cohorts

We conducted a mixed-effects two-way (Sex × Genotype) ANOVA to analyze the gene x environment interaction on Eat-26 scores. This analysis examined both between-group and within-group differences, using Holm Sidak's post-hoc test. Overall, we observed highly significant main effect of sex on *NPSR1* rs324981–related outcomes ($F(3,90) = 31.58$, $p < 0.0001$), whereas neither genotype ($p = 0.6988$) nor the sex × genotype interaction ($p = 0.7793$) reached significance. This indicates that sex differences, rather than genotype class alone, drive the variation observed at this locus. (See Figure 1)

 doi:10.20944/preprints202605.1185.v1

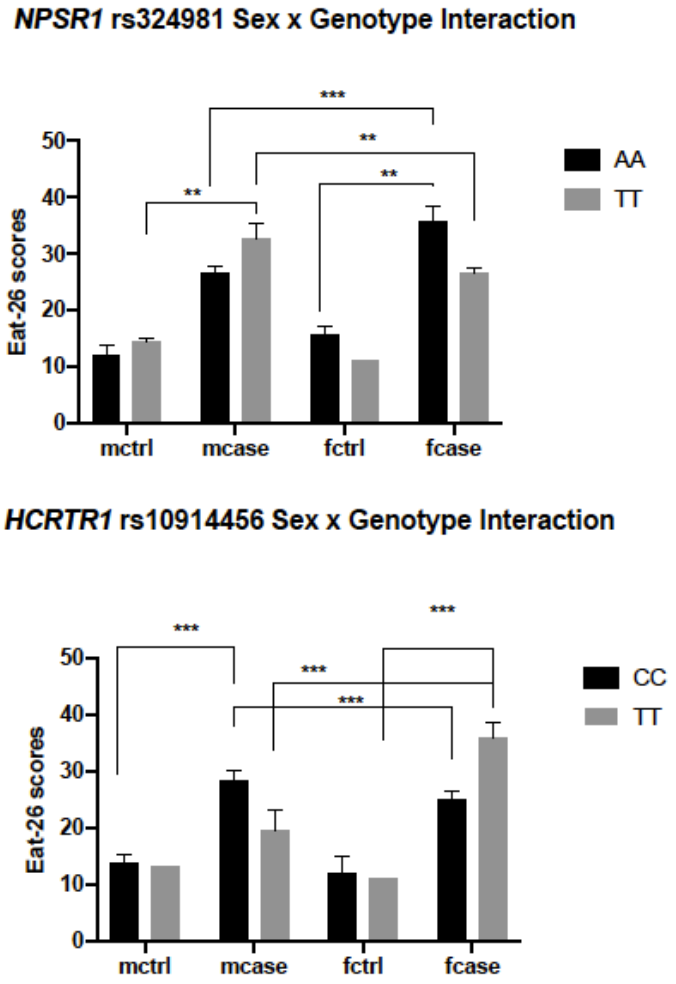

Figure 1. Single nucleotide gene variants *NPSR1* rs324981 and *HCRTR1* rs0914456 , x Eat-26 score interaction, varied by sex. (n=200). Two-way ANOVA Analysis followed by Holm-Sidak's post hoc analysis. (**p<0.01,***p<0.0001,ns=not significant). mctrl=male controls, mcase=male cases, fctrl=female controls, fcases=female cases.

Holm–Šidák post-hoc comparisons showed a consistent and robust elevation in female case values across genotypes. For the *AA* genotype, female cases differed significantly from both male controls (p = 0.001) and female controls (p = 0.02).Similarly, for the *TT* genotype, significant differences were observed between male cases and male controls (p = 0.02) and also against female cases (p = 0.01).

A mixed-effects two-way ANOVA (Sex × Genotype) revealed a highly significant main effect of sex on *HCRTR1* rs10914456–related outcomes ($F(3,54) = 15.15$, $p < 0.0001$), whereas neither genotype ($p = 0.992$) nor the interaction term ($p = 0.972$) reached significance. This indicates that sex differences, rather than genotype class alone, account for the major variance observed at this locus.

Holm–Šidák post-hoc analyses demonstrated a consistent and pronounced elevation among female cases across the common genotypes. Within the CC genotype, male cases differed markedly from male controls (p< 0.0001) and also showed highly significant separation from female cases (p< 0.0001).A similar pattern was seen in the TT genotype, where female cases displayed significantly higher values than both female controls (p = 0.0004) and male cases (p < 0.0001)(see Figure 1).

Overall, these findings highlight a strong sex-driven effect in *HCRTR1* rs10914456 and *NPSR1* rs324981, with female cases showing consistently elevated phenotypic expression across major genotypes (TT and AA) respectively, reinforcing a robust female-specific susceptibility pattern that parallels the allelic and genotypic associations observed in the broader dataset

 doi:10.20944/preprints202605.1185.v1

3.8. Genetic Power and Effect Size Analysis

The case-only sex-stratified power analysis demonstrated clear, well-powered, and biologically coherent sex-specific genetic effects for both *NPSR1 rs324981 and* ***HCRTR1 rs10914456***. For *NPSR1* rs324981, male cases showed a strong T-allele–driven risk effect, with both the heterozygous (AT vs AA) and dominant (AT+TT vs AA) models yielding ORs >3, (OR = 3.00, 95% CI: 1.60–5.00, $p < 0.01$). These effects exceeded the minimum detectable OR of ~2.45 for 80% power, confirming that the male-specific T-allele signal was not only statistically significant but adequately powered and reliably detected.

In contrast, *HCRTR1* rs10914456 exhibited a female-specific recessive genetic effect. The TT genotype was markedly enriched in females, producing a male-vs-female OR of 0.18 (95% CI: 0.06–0.52), equivalent to 5.5-fold higher TT genotype odds in females. This exceeded the recessive model's minimum detectable OR (~3.5–4.5), indicating that the female-specific *HCRTR1* association was statistically robust and well-powered, and unlikely to be a random chance finding. (see Supplementary table).

3.9. Sex Stratified Allele -Dose Logistic Regression:

Allele-dose logistic regression analysis was employed to assess whether, after adjusting for age, BMI, and Eat-26 score, each additional copy of the T allele increases the odds of disease status .Logistic regression using allele-dose coding revealed **sex-specific associations** at both loci , after adjusting for age, BMI, and Eat-26 score. For ***HCRTR1* rs10914456**, female cases carrying the T allele had significantly higher odds of disease risk (OR per T allele = 2.6, 95% CI: 1.20–3.68, $p = 0.009$), whereas no significant association was observed in males (OR = 0.85, 95% CI: 0.42–7.72, $p = 0.65$). For ***NPSR1* rs324981**, male cases carrying the T allele showed markedly increased odds of disease risk (OR = 2.75, 95% CI: 1.55–4.89, $p = 0.001$), whereas in females, the T allele was less frequent in cases (OR = 0.45, 95% CI: 0.22–3.92, $p = 0.028$). Among covariates, **Eat-26 score was positively associated with case status in both sexes** (female: OR = 1.08, 95% CI: 1.02–3.14, $p = 0.008$; male: OR = 1.05, 95% CI: 1.01–5.09, $p = 0.014$), while age and BMI showed no significant effects in males or females ($p>0.05$). These results are consistent with previous genotype-level analyses, confirming a **female-specific recessive effect at *HCRTR1*** and a **male-predominant dominant effect at *NPSR1***, independent of covariates.

4. Discussion

To the best of our knowledge, this is the first comprehensive study providing evidence for sex-specific psycho-social and genetic associations with disordered eating among individuals seeking consultation at nutrition clinics.

Diagnosing eating disorders in Pakistan poses unique challenges due to social and cultural factors. Therefore, in this study, we utilized three tools: the EAT-26, the SCOFF questionnaire, and a self-developed instrument targeting risk factors specific to the Pakistani population. While EAT-26 and SCOFF are internationally recognized tools for diagnosing disorders such as anorexia nervosa and bulimia nervosa [32], their application in Pakistan requires cultural adaptation. To address this, the self-designed questionnaire included culturally relevant risk factors such as media influence, body dissatisfaction, obsession with K-pop body image, academic stress, isolation stress, and peer pressure [33].

The psycho-social findings of this study revealed a consistent, gender-differentiated vulnerability pattern that aligns well with existing literature from Pakistan and across Asia. Although body image dissatisfaction is universally high, stronger female susceptibility to media-driven appearance pressures observed in our study echoes with previous work showing that Pakistani women demonstrate significantly greater thin-ideal internalization and social comparison tendencies than men [34,35]. Similarly, the heightened influence of Korean-pop artists and online beauty standards among females parallels evidence from South Korea, China, and USA, where exposure to

 doi:10.20944/preprints202605.1185.v1

K-pop idols and social media imagery strongly predicts female body dissatisfaction and restrictive eating behaviors [36]. Academic stress was also more strongly linked to disordered eating among females in our sample, nearly consistent with global studies demonstrating that women are more likely to respond to academic pressure with emotional or binge-type eating, whereas men more often adopt external coping strategies [37,38]. Of note, peer-pressure and social comparison effects, reported by nearly four times as many females as males, were also in corroboration of regional data, showing stronger socio-cultural conformity pressures on young Asian women. Thus, increasing their risk for disordered eating trajectories [39,40]. Collectively, these results reinforce that in South Asian cultural contexts, females remain disproportionately affected by media, academic pressure, and peer-driven factors that shape disordered eating patterns.

Moreover, the contrasting inheritance patterns observed across the two loci, point towards , sex-specific mechanisms underlying disordered eating patterns observed in our study population. Although our study was exploratory in nature, we report sex-dependent roles for the *NPSR1* and *HCRTR1* systems in feeding-related phenotypes. Among males, the *NPSR1* rs324981 T-allele showed a dominant pattern, where even a single T allele (AT or TT) was found to be sufficient to markedly elevate vulnerability, whereas among female cases , the AA genotype was relatively more frequent and posed greater risk. These observations are completely in line with our previous report [27] where we found association of *NPSR1* rs324981 TT genotype with increased risk of obesity and also corroborate other human studies showing that the *NPSR1* T-allele heightens stress sensitivity, anxiety traits, and emotional reactivity among males[41,42] and possible interaction with psychosocial stressors to promote maladaptive eating in men as observed in our study. Infact, a neuroimaging-based study, has provided clear evidence of sexually dimorphic effects of *NPSR1* rs324981 [43]. In this study, male patients showed an overrepresentation of the TT genotype with heightened amygdala reactivity and stress sensitivity, whereas females pre-dominantly carried the AA genotype and exhibited distinct prefrontal–limbic activation patterns. These findings demonstrated that *NPSR1* risk pathways diverge markedly by sex, with the T allele conferring risk mainly in males and alternative AA-linked mechanisms shaping vulnerability in females**.** Animal models further support sex-specific *NPSR1* effects on fear, anxiety, and arousal, with engineered mouse models showing diverging behavioral responses by sex after manipulating the human-specific variant rs324981, supporting a biologically plausible male-skewed risk profile in some contexts [44,45].

In contrast, a strong body of murine models-based studies implicate orexin (*HCRTR1*) signaling in binge-like and compulsive palatable-food intake among female rodents. Pre-clinical evidence indicates that female rats display stronger orexinergic signaling, including elevated orexin mRNA, increased *HCRTR1* density, and greater orexin-induced neural activation in feeding and reward circuits (Grafe and Bhatnagar, 2020 ; Zhang et al., 2024). These sex differences translate behaviorally: hypocretin activation promotes stress-induced binge-like eating more powerfully in females [48].

The animal based findings implicate hypocretin/orexin pathway variants in reward/feeding traits and suggest that hypocretin variation might preferentially contribute to female binge-eating phenotypes [47–49]. However, human evidence remains limited, but available studies indicate that genetic variations in *HCRTR*/PPOX (orexin precursor) and *HCRTR1* might influence traits related to impaired self-regulation [50]. However, direct associations between *HCRTR1*-related variants and binge eating disorder in women have not yet been firmly established, as research in this area remains sparse. To date, no human study has demonstrated a clear association between *HCRTR*-system genetic variants and eating disorder pathology in women.

The present study is therefore the first to provide evidence that *HCRTR1* rs10914456 confers a strong, sex-specific genetic risk, with the recessive TT genotype markedly elevating vulnerability in females. Animal studies have repeatedly shown that female rodents exhibit greater hypocretin system -induced locomotor activation, reward sensitivity, and stress-arousal coupling compared to males [46]. In this context, the female-linked genotype patterns observed for *HCRTR1* in our dataset converge with the broader literature, suggesting that orexin-mediated arousal and reward pathways

 doi:10.20944/preprints202605.1185.v1

interact with female hormonal and stress systems to heighten susceptibility to binge-eating behaviors.

These findings introduce a novel biological pathway for female-predominant eating disorder risk and have filled a critical gap in the existing neurogenetic literature. Although the limited sample size warrants replication in larger cohorts.

Of note , multiple genetic systems, including serotonergic, dopaminergic, and other neuropeptides (melanocorticoids and leptins), have been more consistently implicated in the regulation of appetite, reward, and energy balance [51]. Therefore, the observed associations in *NPSR1* and *HCRTR1* might reflect a modulatory role of these neuropeptide systems, potentially interacting with these other genetic and environmental factors. Hence , future studies should be based on understanding the impact of these novel associations in context of these neurotransmitter systems with well- established roles in eating disorders.

The converging psychometric and genetic signals point to sex-specific pathways into eating pathology. Females' higher EAT-26 burden alongside stronger links with media, academic pressure, and appearance-based comparison, suggests that socio-cultural stressors are not merely correlates but likely to amplify vulnerability.

When viewed alongside the *NPSR1* findings, a consistent picture emerges: both hypocretin and Neuropeptide S pathways converge on overlapping stress–arousal networks implicated in the regulation of appetite and emotional eating. The sex-dependent differences observed revealed stronger hypocretin-related effects in females and greater *NPSR1*-driven vulnerability in males further highlighting the importance of neuroendocrine context, suggesting that hormonal modulation may shape how these neuropeptide systems contribute to eating-related psychopathology. Although, the study was exploratory in nature and warrants validation in larger sample sets. Collectively, these data sets advance the understanding of gene–behavior interactions in disordered eating and provide translational insight into how sex-informed, neuropeptide-targeted interventions could be designed to mitigate risk across metabolic and psychiatric domains.

The present findings should be interpreted in the context of emerging genetic research on eating disorders and related psychiatric phenotypes in South Asian populations, where data remains limited. Epidemiological studies from Pakistan[52] and the broader region [53] indicate that disordered eating behaviors are prevalent and influenced by sociocultural and psychological factors. However, genetic studies in this domain remain scarce. Importantly, population-genetic research has demonstrated that South Asian populations exhibit substantial genetic heterogeneity, including high levels of homozygosity and distinct allele frequency distributions compared to European populations [54]. These features may contribute to differences in genetic effect sizes and the detection of recessive or sex-specific associations. Therefore, the observed sex- and locus-specific effects in the present study may reflect both biological mechanisms and population-specific genetic architecture, underscoring the need for further replication in a larger South Asian cohort. Overall, our observations of sex- and locus-specific effects at *HCRTR1* rs10914456 and *NPSR1 rs324981* are consistent with the broader literature highlighting heterogeneity in genetic susceptibility across populations and sexes, although direct comparisons are constrained by the limited number of studies in comparable cohorts. It is also important to consider the influence of sample size on effect estimates. Therefore, to mitigate the impact of modest sample size, which might contribute to overestimation of effect sizes, we conducted multiple complementary analyses, including allele-dose regression analysis , dominant, and recessive models, to ensure consistency of findings. While the consistency of effects across allele-dose and genotype-level models supports the robustness of the findings, replication in larger, independent cohorts is necessary to confirm the magnitude and direction of these associations. Furthermore, although key covariates such as age, BMI, and Eat-26 score were included in the adjusted models, additional biological confounders were not assessed. In particular, hormonal status, menstrual cycle phase, and fluctuations of sex hormone may influence both neuropeptide signaling pathways and behavioral phenotypes, potentially contributing to the observed sex-specific effects. Future studies

 doi:10.20944/preprints202605.1185.v1

incorporating endocrine measures and longitudinal designs would be valuable in disentangling these interactions and refining the interpretation of sex-dependent genetic associations.

From a clinical and prevention standpoint, a stepped approach (SCOFF→EAT-26) appears effective for initial assessment, and the risk patterns indicate the need for strategies tailored to each sex. For females, programs that combine media literacy and stress management may offer the most benefits; for males, focusing on pleasure eating and peer influences may be crucial.

4.1. Strengths and Limitations of the Study

This study, while novel, is not without limitations. The cross-sectional design precludes causal inference, and the sample size was modest . However, we strengthened the validity of our findings by conducting a comprehensive post-hoc genetic power analysis, the results of which confirmed that the associations we observed are statistically robust and not artifacts of limited sample size (see Supplementary files). Recruitment challenges, particularly among women who were hesitant to participate due to cultural sensitivities around both mental health investigations and genetic analysis, also shaped the scope of our study. Consequently, we used a cross-sectional sample of participants visiting clinics and were able to probe genetic association for only two SNVs. Moreover, participants were recruited from clinical settings where individuals were already seeking consultation for eating disorders or weight-related concerns. Consequently, the proportion of participants scoring ≥ 20 on the EAT-26 might represent an overestimation compared with prevalence estimates in the general population. Furthermore, the sample largely represents an urban population with a mean age of 28.6 years, and the gender distribution was intentionally balanced for comparative purposes. Therefore, the results may not be fully representative of the broader Pakistani population. Future studies using larger, population-based samples are required to validate these findings. Despite these constraints, the willingness of participants to share their experiences highlights the urgent need to expand such research. Future studies should incorporate larger, longitudinal cohorts to better unravel the interplay between genetic predispositions and modifiable behavioral factors in shaping eating disorder outcomes.

5. Conclusions

Together, these data delineate a dual-pathway model of sex-specific genetic vulnerability, in which *NPSR1* T-allele–driven arousal and anxiety sensitivity predominantly heighten risk in males, while enhanced Hypocretin system responsivity amplifies compulsive, stress-reactive eating predominantly in women, yet with distinct genotype-specific patterns emerging across loci. The convergence of robust statistical evidence, biological plausibility, and cross-species mechanistic support underscores the importance of incorporating sex as a central biological variable in genetic studies of eating-disorder phenotypes. These findings highlight the need for sex-stratified therapeutic strategies targeting neuropeptide systems implicated in stress, reward, and arousal regulation.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Information: Genetic Power and Effect Size Analysis; Table S1: Genetic power and effect size analysis for the SNVs *NPSR1* rs324981 and *HCRTR1* rs10914456.p-value was set at <0.05.

Author Contributions: PG designed and supervised the study, analyzed data, performed experiments and wrote manuscript , KA performed experiments and curated data sets.

Funding: This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

Institutional Review Board Statement: The study adhered to the Helsinki Declaration of 1975 (revised in 2013) and all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and was approved by the Ethics Review Board of Comsats University Islamabad (CUI/BIO/ERB/2024/41). The authors assert that all procedures contributing to this work

 doi:10.20944/preprints202605.1185.v1

comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2013.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Acknowledgments: The authors would like to thank all the participants for their participation and cooperation.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Barakat, S., McLean, S.A., Bryant, E. et al. (2023)Risk factors for eating disorders: findings from a rapid review. J Eat Disord 11, 8. https://doi.org/10.1186/s40337-022-00717-4.
2. Fairburn CG, Walsh BT (2002). Atypical eating disorders. In: Fairburn CG, Brownell KD, editors. Eating disorders and obesity: a comprehensive textbook. New York: Guilford Press;. p. 171– 77.
3. Diagnostic and statistical manual of mental disorders. 4th ed.: American Psychiatric Association. Washington (DC), 1994;154.
4. Herzog, D. B., Dorer, D. J., Keel, P. K., Selwyn, S. E., Ekeblad, E. R., Flores, A. T., Greenwood, D. N., Burwell, R. A., & Keller, M. B. (1999). Recovery and relapse in anorexia and bulimia nervosa: a 7.5-year follow-up study. Journal of the American Academy of Child and Adolescent Psychiatry, 38(7), 829–837. https://doi.org/10.1097/00004583-199907000-00012.
5. Hamilton A, Mitchison D, Basten C, Byrne S, Goldstein M, Hay P, et al. 2021.Understanding treatment delay: perceived barriers preventing treatment-seeking for eating disorders. Aust N Z J Psychiatry. 2021;12:00048674211020102. doi: 10.1177/00048674211020102
6. Culbert, K. M., Sisk, C. L., & Klump, K. L. (2021). A Narrative Review of Sex Differences in Eating Disorders: Is There a Biological Basis?. Clinical therapeutics, 43(1), 95–111. https://doi.org/10.1016/j.clinthera.2020.12.003
7. Schmidt, U., Adan, R., Böhm, I., Campbell, I. C., Dingemans, A., Ehrlich, S., Elzakkers, I., Favaro, A., Giel, K., Harrison, A., Himmerich, H., Hoek, H. W., Herpertz-Dahlmann, B., Kas, M. J., Seitz, J., Smeets, P., Sternheim, L., Tenconi, E., van Elburg, A., Zipfel, S. (2016). Eating disorders: The big issue. *The Lancet Psychiatry*, *3*(4), 313–315. https://doi.org/10.1016/S2215-0366(16)00081-X
8. .Zahra S. M., Jha, R. P., Safdar, M., Khalid, M. Z., Khalid, W., & Ranjha, M. M. A. N. (2023). Trends in the Burden of Eating Disorders in Pakistan over the Past Three Decades: A Joinpoint Regression Analysis. Annals of Indian Psychiatry, 7(2), 140-151. Doi:10.4103/aip.aip_8_22
9. López-Gil JF, García-Hermoso A, Smith L, et al (2023). Global Proportion of Disordered Eating in Children and Adolescents: A Systematic Review and Meta-analysis . JAMA Pediatr. ;177(4):363–372. doi:10.1001/jamapediatrics.2022.5848
10. Javaid, Fasiha, et al. (2020) "Determinants of eating habits and physical activity among female students of government schools of urban city of Pakistan." Journal of Ayub Medical College Abbottabad 32.4: 517-522.https://jamc.ayubmed.edu.pk/index.php/jamc/article/view/8205
11. Zafar, H., & Mobin, M. B. (2024). Excessive usage of social media: A potential threat to mental health in Pakistan. Journal of Pakistan Medical Association, 74(1), 201-201. https://doi.org/10.47391/JPMA.9907
12. Yoon, K. (2022). Beneath the Surface: The Struggles of Dismantling Lookism in Looks Obsessed Korea. Embodied: The Stanford Undergraduate Journal of Feminist, Gender, and Sexuality Studies, 1(1).
13. Memon, A. A., Adil, S. E. E. R., Siddiqui, E. U., Naeem, S. S., Ali, S. A., & Mehmood, K. (2012). Eating disorders in medical students of Karachi, Pakistan-a cross-sectional study. BMC research notes, 5, 1-7.DOI: 10.1186/1756-0500-5-84
14. Jahrami, H., Sater, M., Abdulla, A., Faris, M. E. A. I., & AlAnsari, A. (2019). Eating disorders risk among medical students: a global systematic review and meta-analysis. Eating and Weight Disorders-Studies on Anorexia, Bulimia and Obesity, 24, 397-410. DOI: 10.1007/s40519-018-0516-z

 doi:10.20944/preprints202605.1185.v1

15. Donato K, Ceccarini MR, Dhuli K, Bonetti G, Medori MC, Marceddu G, Precone V, Xhufi S, Bushati M, Bozo D, Beccari T, Bertelli M. Gene variants in eating disorders (2022). Focus on anorexia nervosa, bulimia nervosa, and binge-eating disorder. J Prev Med Hyg. Oct 17;63(2 Suppl 3):E297-E305. doi: 10.15167/2421-4248/jpmh2022.63.2S3.2772. PMID: 36479493; PMCID: PMC9710388
16. Mayhew, A. J., Pigeyre, M., Couturier, J., & Meyre, D. (2018). An Evolutionary Genetic Perspective of Eating Disorders. Neuroendocrinology, 106(3), 292–306. https://doi.org/10.1159/000484525
17. Jimerson DC, Wolfe BE. Neuropeptides in eating disorders. CNS Spectr. 2004 Jul;9(7):516-22. doi: 10.1017/s1092852900009603. PMID: 15208511
18. Jacobson LH, Hoyer D, de Lecea L. Hypocretins (orexins): The ultimate translational neuropeptides. J Intern Med. 2022 May;291(5):533-556. doi: 10.1111/joim.13406. Epub 2022 Jan 19. PMID: 35043499.
19. Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R. M., Tanaka, H., ... & Yanagisawa, M. (1998). Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell*, 92(4), 573–585. [https://doi.org/10.1016/S0092-8674(00)80949-6].
20. Chao, A. M., Wadden, T. A., & Walsh, B. T. (2011). The role of orexin in the pathophysiology of binge eating. CNS Spectrums, 16(6), 381–386. [https://doi.org/10.1017/S1092852912000321]
21. Haynes, A. C., Jackson, B., Overend, P., Buckingham, R. E., Wilson, S., & Tadayyon, M. (2002). Effects of single orexin-A administration on food intake and feeding behaviour in the rat. Endocrinology, 143(2), 562–569.[https://doi.org/10.1210/endo.143.2.8615]
22. Beck, B.; Fernette, B.; Stricker-Krongrad A. Peptide S is a novel potent inhibitor of voluntary and fast-induced food intake in rats. Biochem. Biophys. Res. Commun., 2005, 332, 859-865. DOI: 10.1016/j.bbrc.2005.05.029
23. Cifani C, Micioni Di Bonaventura MV , Cannella N, Fedeli A, Guerrini R, Calo G, Ciccocioppo R, Ubaldi M.Effect of neuropeptide S receptor antagonists and partial agonists on palatable food consumption in the rat. Peptides, 2011; 32; 44-50. https://doi.org/10.1016/j.peptides.2010.10.018
24. Xu, Y. L., Reinscheid, R. K., Huitron-Resendiz, S., Clark, S. D., Wang, Z., Lin, S. H., ... & Civelli, O. (2004). Neuropeptide S: a neuropeptide promoting arousal and anxiolytic-like effects. Neuron, 43(4), 487–497. [https://doi.org/10.1016/j.neuron.2004.08.005]
25. Raczka, K. A., Mechias, M. L., Gartmann, N., Reif, A., Büchel, C., Deckert, J., & Kalisch, R. (2010). Empirical support for an involvement of the NPS system in anxiety and fear regulation in humans. *Neuropsychopharmacology*, 35(4), 911–920. https://doi.org/10.1038/npp.2009.197.
26. Ghazal P (2016). The physio-pharmacological role of Neuropeptide S in psychiatric disorders: A translational overview. Current protein and peptide science, 17, 1-18. doi: 10.2174/1389203717666151218150704.
27. Ahmad A, Almsned F, Ghazal P, Ahmed MW, Jafri MS, Bokhari H (2020) Neuropeptide S receptor gene Asn107 polymorphism in obese male individuals in Pakistan. PLoS ONE 15(12): e0243205. https://doi.org/10.1371/journal.pone.0243205
28. Cengiz M, Karaj V, Kocabasoğlu N, Gozubatik-Celik G, Dirican A, Bayoglu B.(2019). Orexin/hypocretin receptor, HCRTR1, gene variants are associated with major depressive disorder. Int J Psychiatry Clin Pract. Jun;23(2):114-121. doi: 10.1080/13651501.2018.1551549. Epub 2018 Dec 31. PMID: 30596528.
29. Morgan JF, Reid F, Lacey JH (2000). The SCOFF questionnaire: a new screening tool for eating disorders. West J Med. Mar;172(3):164-5. doi: 10.1136/ewjm.172.3.164. PMID: 18751246; PMCID: PMC1070794.
30. Garner, D. M., Olmsted, M. P., Bohr, Y., & Garfinkel, P. E. (1982). The eating attitudes test: psychometric features and clinical correlates. Psychological medicine, 12(4), 871–878. https://doi.org/10.1017/s0033291700049163
31. Miller SA, Dykes DD, Polesky HF, (1988).A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. Feb 11;16(3):1215. doi: 10.1093/nar/16.3.1215. PMID: 3344216; PMCID: PMC334765.
32. Schaefer LM, Crosby RD, Machado PPP. A systematic review of instruments for the assessment of eating disorders among adults. Curr Opin Psychiatry. 2021 Nov 1;34(6):543-562. doi: 10.1097/YCO.0000000000000746. PMID: 34475351; PMCID: PMC8645259.

 doi:10.20944/preprints202605.1185.v1

33. Abbas J, Aman J, Nurunnabi M, Bano S 2019. The Impact of Social Media on Learning Behavior for Sustainable Education: Evidence of Students from Selected Universities in Pakistan. Sustainability.; 11(6):1683. https://doi.org/10.3390/su11061683.
34. Khan R, Bibi H , Naz, S & Khan F. (2023). Body Surveillance and Body Dissatisfaction among Pakistani Young Females: Social Comparison as Moderator. Human Nature Journal of Social Sciences. 4. 16-26. https://doi.org/10.71016/hnjss/nc0e0c32
35. Saeed, M., Afzal, H., Khawar, H., Khan, Z. K., Idrees, S., & Maqbool, H. S. (2023). Role of social media in comparing physical appearance, body dissatisfaction and drive for thinness. Bulletin of Business and Economics, 14(1), 6–11. https://doi.org/10.61506/01.00571
36. Jung, J., & Forbes, G. B. (2007). Body dissatisfaction and disordered eating among college women in China, South Korea, and the United States: Contrasting predictions from sociocultural and feminist theories. Psychology of Women Quarterly, 31(4), 381–393. https://doi.org/10.1111/j.1471-6402.2007.00387.x
37. Graves BS, Hall ME, Dias-Karch C, Haischer MH, Apter C (2021) Gender differences in perceived stress and coping among college students. PLoS ONE 16(8): e0255634. https://doi.org/10.1371/journal.pone.0255634
38. Vidic, Z. Gender differences on coping, stress, resilience and mindfulness within an academic course intervention with a mindfulness meditation component. Curr Psychol 43, 28241–28251 (2024). https://doi.org/10.1007/s12144-024-06395-6
39. Akoury LM, Warren CS and Culbert KM (2019) Disordered Eating in Asian American Women: Sociocultural and Culture-Specific Predictors. Front. Psychol. 10:1950. doi: 10.3389/fpsyg.2019.01950
40. Han X, Cheung MC, Corcoran J. Family functioning and eating disorders in Chinese populations: a systematic review and meta-synthesis. J Eat Disord. 2025 Nov 24;13(1):269. doi: 10.1186/s40337-025-01453-1. PMID: 41287111; PMCID: PMC12641987.
41. Domschke K, Reif A, Weber H, Richter J, Hohoff C, Ohrmann P, Pedersen A, Bauer J, Suslow T, Kugel H, Heindel W, Baumann C, Klauke B, Jacob C, Maier W, Fritze J, Bandelow B, Krakowitzky P, Rothermundt M, Erhardt A, Binder EB, Holsboer F, Gerlach AL, Kircher T, Lang T, Alpers GW, Ströhle A, Fehm L, Gloster AT, Wittchen HU, Arolt V, Pauli P, Hamm A, Deckert J. Neuropeptide S receptor gene -- converging evidence for a role in panic disorder. Mol Psychiatry. 2011 Sep;16(9):938-48. doi: 10.1038/mp.2010.81. Epub 2010 Jul 6. PMID: 20603625.
42. Laas K, Reif A, Akkermann K, Kiive E, Domschke K, Lesch KP, Veidebaum T, Harro J (2015). Neuropeptide S receptor gene variant and environment: contribution to alcohol use disorders and alcohol consumption. Addict Biol. May;20(3):605-16. doi: 10.1111/adb.12149. Epub 2014 Apr 23. PMID: 24754478.
43. Laas K, Reif A, Kiive E, Domschke K, Lesch KP, Veidebaum T, Harro J (2014). A functional NPSR1 gene variant and environment shape personality and impulsive action: a longitudinal study. J Psychopharmacol. Mar;28(3):227-36. doi: 10.1177/0269881112472562. Epub 2013 Jan 16. PMID: 23325374.
44. Song C, Zhu ZC, Liu CC, Yun WX, Wang ZY, Lu GY, Song R, Wu N, Li J, Li F. Neuropeptide S Receptor 1 variant (I107N) regulates behavioral characteristics and NPS effect in mice in a sex-dependent manner. Neuropharmacology. 2024 Jan 1;242:109771. doi: 10.1016/j.neuropharm.2023.109771. Epub 2023 Oct 17. PMID: 37858885.
45. Streit F, Akdeniz C, Haddad L, Kumsta R, Entringer S, Frank J, Yim IS, Zänkert S, Witt SH, Kirsch P, Rietschel M, Wüst S. Sex-specific association between functional neuropeptide S receptor gene (NPSR1) variants and cortisol and central stress responses. Psychoneuroendocrinology. 2017 Feb;76:49-56. doi: 10.1016/j.psyneuen.2016.10.027. Epub 2016 Nov 10. PMID: 27883964
46. Grafe LA, Bhatnagar S. The contribution of orexins to sex differences in the stress response. Brain Res. 2020 Mar 15;1731:145893. doi: 10.1016/j.brainres.2018.07.026. Epub 2018 Aug 3. PMID: 30081036; PMCID: PMC6360123.
47. Zhang J, Jin K, Chen B, Cheng S, Jin J, Yang X, Lu J, Song Q. Sex-dimorphic functions of orexin in neuropsychiatric disorders. Heliyon. 2024 Aug 15;10(16):e36402. doi: 10.1016/j.heliyon.2024.e36402. PMID: 39253145; PMCID: PMC11382083.

 doi:10.20944/preprints202605.1185.v1

48. Freeman LR, Bentzley BS, James MH, Aston-Jones G. Sex Differences in Demand for Highly Palatable Foods: Role of the Orexin System. Int J Neuropsychopharmacol. 2021 Jan 20;24(1):54-63. doi: 10.1093/ijnp/pyaa040. PMID: 32496559; PMCID: PMC7816693
49. Piccoli L, Micioni Di Bonaventura MV, Cifani C, Costantini VJ, Massagrande M, Montanari D, Martinelli P, Antolini M, Ciccocioppo R, Massi M, Merlo-Pich E, Di Fabio R, Corsi M. Role of orexin-1 receptor mechanisms on compulsive food consumption in a model of binge eating in female rats. Neuropsychopharmacology. 2012 Aug;37(9):1999-2011. doi: 10.1038/npp.2012.48. Epub 2012 May 9. PMID: 22569505; PMCID: PMC3398727.
50. Aliev, F., De Sa Nogueira, D., Aston-Jones, G. et al. Genetic associations between orexin genes and phenotypes related to behavioral regulation in humans, including substance use. Mol Psychiatry 30, 2922–2930 (2025). https://doi.org/10.1038/s41380-025-02895-4
51. Bulik, C.M., Coleman, J.R.I., Hardaway, J.A. et al. Genetics and neurobiology of eating disorders. *Nat Neurosci* **25**, 543–554 (2022). https://doi.org/10.1038/s41593-022-01071-
52. Suhail K, Zaib-u-Nisa. Prevalence of eating disorders in Pakistan: relationship with depression and body shape. Eat Weight Disord. 2002 Jun;7(2):131-8. doi: 10.1007/BF03354439. PMID: 17644867.
53. Pengpid S, Peltzer K. Risk of disordered eating attitudes and its relation to mental health among university students in ASEAN. Eat Weight Disord. 2018 Jun;23(3):349-355. doi: 10.1007/s40519-018-0507-0. Epub 2018 Apr 21. PMID: 29681011.
54. Wall, J.D., Sathirapongsasuti, J.F., Gupta, R. et al. South Asian medical cohorts reveal strong founder effects and high rates of homozygosity. *Nat Commun* **14**, 3377 (2023). https://doi.org/10.1038/s41467-023-38766-1