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TESIS DOCTORAL

"ESTUDIO DE LA INFLUENCIA DE LA VARIABILIDAD GENÉTICA EN GENES DE OBESIDAD SOBRE LA ETIOPATOGENIA DE LOS TRASTORNOS DE LA ALIMENTACIÓN"

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Que Dª Carmen Gamero Vilarroel, Licenciada en Bioquímica, ha realizado bajo su dirección el presente trabajo titulado "*Estudio de la influencia de la variabilidad genética en genes de obesidad sobre la etiopatogenia de los trastornos de la alimentación*"

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ABREVIATURAS

5-HT	Serotonina
A	Ascetismo, del inglés Asceticism
ACTH	Hormona adrenocorticotropa
ADN	Ácido desoxirribonucleico
AGRP	Proteína relacionada con Agouti
AN	Anorexia Nerviosa
ANCP	Anorexia Nerviosa tipo compulsivo/purgativo
ANR	Anorexia Nerviosa tipo restrictivo
B	Bulimia
BD	Insatisfacción corporal, del inglés body dissatisfaction
BDNF	Factor Neurotrófico derivado del cerebro
BN	Bulimia Nerviosa
CCK	Colecistoquinina
COMT	Catecol-O-metil-transferasa
DRD2	Gen del receptor de dopamina D2
DRD3	Gen del receptor de dopamina D3
DRD4	Gen del receptor de dopamina D4
DSM IV	Manual Diagnóstico y Estadístico de los Trastornos Mentales, cuarta edición.

DSM V	Manual Diagnóstico y Estadístico de los Trastornos Mentales, quinta edición.
DT	Obsesión por la delgadez, del inglés drive for thinness
EDI-2	Inventario de los trastornos de la conducta alimentaria del inglés, *Eating Disorders Inventory Test-2*
ESR2	Receptor estrogénico β
GPI	Glicofosfatidilinositol
GSI	Índice de Severidad Global, del inglés Global Severity Index
GWAS	del inglés Genome-wide Association Studies
HSCL	Hopkins Symptom Checklist
I	Ineficacia, del inglés Ineffectiveness
IA	Conciencia Interoceptiva, del inglés Interoceptive Awareness,
IA	Ingesta por atracón
IC	Intervalo de confianza
ID	Desconfianza Interpersonal, del inglés Interpersonal Distrust,
IMC	Índice de Masa Corporal
IR	Impulsividad, del inglés Impulse Regulation,
KCCN3	Canal SK3
LAMP	Proteína de membrana asociada al sistema límbico
MC4R	Receptor de melacortina 4
MF	Miedo a la Madurez, del inglés Maturity Fears,

MSH	Hormona Estimulante de Melanocito
NE	Norepinefrina
NEGR1	Regulador de crecimiento neuronal 1
NMDA	Glutamato
NPY	Neuropéptido Y
Ntm	Neurotrimina
OB	Obesidad
OBCAM	Molécula de adhesión celular de unión a opioides
OR	Odds Ratio
P	Perfeccionismo, del inglés Perfectionism
PCR	Reacción en cadena de la polimerasa
POMC	Proopiomelacortina
PSDI	Índice Positivo de Malestar, del inglés Positive Symptom Distress Index
PST	Total de Síntomas Positivos, del inglés Positive Symptom Total,
SCL-90R	Inventario de 90 síntomas revisado, del inglés *Symptom Checklist 90 Revisado*
SI	Inseguridad Social, del inglés Social Insecurity
SNC	Sistema Nervioso Central
SNP	Polimorfismo de un solo nucleótido
TA	Trastorno por Atracón

TCA	Trastornos de la Conducta Alimentaria
TCANE	Trastornos de la Conducta Alimentaria no Especificados.
TNF- α	Factor de necrosis tumoral alfa
TrkB	Receptor tropomiosin kinasa tipo B
UCP2	Proteína desacoplante 2
UCP3	Proteína desacoplante 3

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1. RESUMEN

1. RESUMEN

En los últimos años se han identificado una serie de proteínas, neurotransmisores y hormonas capaces de regular la conducta alimentaria y/o de modular las rasgos psicopatológicos que a menudo presentan los pacientes con trastornos alimentarios. Entre ellos destacan genes de expresión hipotalámica implicados en las vías centrales de regulación de la ingesta y que además se han asociado con la obesidad o con el índice de masa corporal (IMC). Varios trabajos han centrado su atención en el estudio de la variabilidad del gen *BDNF* debido a su destacado papel en el desarrollo neuronal y a su participación en el control del balance energético y en los centros de recompensa del cerebro. Por otro lado, el gen *NEGR1* ha sido identificado en numerosos estudios GWAS (del inglés *Genome Wide Associated Studies*, estudios de asociación del genoma completo) como un gen asociado con la obesidad. Aunque hasta la fecha su función exacta es desconocida, parece desempeñar un papel clave en el control neuronal del peso y la ingesta. Finalmente, el gen *MC4R*, por su implicación en el sistema leptina-melanocortina, también ha sido ampliamente estudiado en el campo de la obesidad; de hecho, las mutaciones en este gen han sido consideradas como la principal causa de obesidad monogénica en humanos.

El objetivo del presente trabajo fue establecer si la existencia de polimorfismos en los genes anteriormente citados, relacionados con los mecanismos de regulación de la ingesta, puede influenciar el riesgo a desarrollar un Trastorno de la Conducta Alimentaria (TCA) y/o modular los parámetros fisiológicos y psicopatológicos en estos pacientes. Para ello analizamos 6 polimorfismos del gen *BDNF* y 21 del gen *NEGR1* en 169 mujeres con diagnóstico de TCA (106 con Anorexia Nerviosa, AN y 63 con bulimia nerviosa, BN) y 312 controles sanos. Además analizamos la secuencia completa del gen *MC4R* en tres grupos de estudio (170 pacientes con obesidad, 77 mujeres no obesas con comportamiento por atracón con diagnóstico de BN o de Trastorno por Atracón (TA) y 20 mujeres con AN).

Nuestros resultados muestran que ninguno de los SNPs (del inglés *single nucleotide polimorfism*) o haplotipos analizados de los genes *BDNF* y *NEGR1* desempeñaba un papel relevante en el riesgo de padecer un TCA. Sin embargo el genotipo TT del SNP rs16917237 del gen *BDNF* se asociaba con un aumento del peso y del IMC y la presencia de dos haplotipos del locus 3 (rs10835210 / rs16917237 / C-270T) del gen mostraba un marcado efecto sobre las puntuaciones del cuestionario SCL-90R. En cuanto al estudio del gen *NEGR1* destacó la presencia de un bloque haplotípico formado por los SNPs rs12740031, rs10789322, rs954299 y rs2422021 relacionado con las puntuaciones totales del cuestionario EDI-2 en pacientes con BN. Este bloque se incluía dentro de una región central del gen asociada a su vez, con mayores puntaciones de las escalas *Obsesión por la delgadez*, *Ineficacia*, *Conciencia Interoceptiva* y *Bulimia* en pacientes con BN. Finalmente, se identificaron 10 variantes del gen *MC4R* en pacientes obesos, mientras que tan solo dos pacientes con episodios de purga y atracón eran portadoras del polimorfismo I125L. Estas dos pacientes no presentaban valores de peso o características psicopatológicas diferentes de las pacientes no portadoras.

Nuestros resultados permiten concluir que ciertos polimorfismos de los genes *BDNF* y *NEGR1* pueden tener una influencia relevante en determinados rasgos de la personalidad característicos de pacientes con TCA.

Palabras clave: trastornos de la conducta alimentaria, anorexia, bulimia, *BDNF, NEGR1, MC4R*

SUMMARY

In the past years, researchers have identified a number of proteins, neurotransmitters and hormones with the ability to regulate eating behavior and/or modulate the psychopathological traits that are often present in patients with eating disorders (ED). Among them, those with expression within hypothalamic pathways involved in the regulation of food intake and/or associated with obesity or body mass index (BMI) have been pointed out as good candidates in genetic research on ED.

Several studies have focused on *BDNF* genetic variability because of its prominent role in neuronal development and its involvement in energy balance and reward centers of the brain. On the other hand, the *NEGR1* gene has been identified as an important obesity locus in numerous GWAS (Genome Wide Associated Studies). Although the specific function of the gene is presently unknown, it seems likely that it plays a key role in the neuronal control of body weight and food intake. Finally, the *MC4R* gene, because of its involvement in the leptin-melanocortin system, has been intensively analyzed in the research of obesity genetics; indeed, *MC4R* mutations constitute the most common monogenic cause of human obesity.

The aim of this study was to establish whether the existence of polymorphisms in above mentioned genes, involved in the neuronal control of weight may also influence in the risk to develop a ED and/or modulate physiological or psychopathological parameters in these patients. For this, we analyzed 6 polymorphisms in *BDNF* and 21 in *NEGR1* in 169 women diagnosed of ED (106 with Anorexia nervosa, AN and 63 with bulimia nervosa, BN) and 312 healthy controls. We also screened the complete coding region of the *MC4R* gene in three study groups (170 obese patients, 77 non-obese women with binge eating behavior diagnosed with BN or Binge Eating Disorder and 20 women with AN).

Our results show that none of the *BDNF* and *NEGR1* SNPs or haplotypes analyzed were associated with a greater risk of ED. However, the TT genotype of rs16917237 *BDNF* SNP was associated with higher weight and BMI and two haplotypes in locus 3 (rs10835210 / rs16917237 / C-270T) showed a broad effect on the scores of SCL-90R test. On the other hand, the study of the *NEGR1* gen pointed out the presence of an haplotype block formed by the rs12740031, rs10789322, rs954299 and rs2422021 SNPs that was associated with total scores of EDI-2 test in BN patients. This block was included within a central region, which in turn was associated with higher scores in the drive for thinness, inefficiency, bulimia and interoceptive awarness scales in BN patients. Finally, 10 different variants of *MC4R* were identified in the obesity group, whilst in the binge-eating patients only two individuals with BN were found to carry the I251L polymorphism, which did not correlate with weight or psychopatological features

Our results suggest that certain polymorphisms of the *BDNF* and *NEGR1* genes could have influence in certain personality traits of patients with eating disorders.

Keywords: Eating disorders, anorexia, bulimia, BDNF, NEGR1, MC4R.

2. INTRODUCCIÓN

2.1. LOS TRASTORNOS DE LA CONDUCTA ALIMENTARIA

2.1.1. Concepto de Trastorno de la Conducta Alimentaria

Los trastornos de la conducta alimentaria (TCA) son enfermedades psiquiátricas que han proliferado en los últimos años, llegando a convertirse hoy en día, en la tercera enfermedad crónica entre la población femenina adolescente y juvenil en las sociedades desarrolladas y occidentalizadas (Peláez, 2005). Su creciente interés es debido a que se asocian con baja calidad de vida, altas tasas de comorbilidad psicosocial y mortalidad prematura y predisponen a los pacientes a situaciones de obesidad y desnutrición (Herpertz-Dahlmann, 2009). Por otra parte, su tratamiento requiere la actuación de un equipo multidisciplinario, que conlleva ingresos hospitalarios de larga duración, lo que comporta un gran consumo de recursos (Bergh y Sodersten, 1998). El aumento en las cifras de incidencia de patologías como la *anorexia nerviosa* (AN) y la *bulimia nerviosa* (BN) y otros trastornos más inespecíficos, ha incrementado el número de estudios de investigación en relación a la etiopatogenia, predisposición, tratamiento y evolución de estos trastornos.

En los últimos años se ha conseguido avanzar en el conocimiento de los TCA, convirtiéndose en la actualidad en categorías consolidadas dentro de las clasificaciones internacionales de las enfermedades mentales. Además de la AN y BN, otras alteraciones alimentarias, como el denominado *trastorno por atracón* (del inglés *binge-eating disorder,* TA), han sido incluidas en el DSM IV (A.P.A., 1994) aunque solamente como categorías diagnósticas potenciales. Recientemente se ha publicado el DSM V (A.P.A., 2013) pero en este trabajo nos referiremos a los criterios DSM IV, al no estar definidos los criterios del DSM V cuando al inicio del estudio.

2.1.2. Epidemiología

Los TCA se desarrollan fundamentalmente durante la adolescencia o juventud, aunque existen casos excepcionales que pueden aparecer después de los 40 años o en la infancia (García-Camba, 2001). Las tasas de incidencia de AN son mayores en mujeres jóvenes de entre 15 y 19 años y llegan a constituir el 40% de los nuevos casos diagnosticados (Hoek, 2006). La BN tienen un comienzo algo más tardío, alrededor de los 18 o 20 años, a lo cual contribuye el hecho de que un número importantes de casos de BN, se desarrollan en pacientes que han padecido AN previamente (García-Camba, 2001).

Según la American Psychological Association (A.P.A.) y revisiones recientes de estudios epidemiológicos de trastornos alimentarios (Hoek y van Hoeken, 2003), se calcula que en mujeres jóvenes y adolescentes de países occidentales, existe una prevalencia de entre el 0,5 y 1% de AN, 1 y 3% de BN y aproximadamente entre 3 y 5% de TCA no especificados (TCANE).

Un estudio reciente sitúa la incidencia en AN en 8 casos nuevos/100.000 personas/año. (Hoek, 2006). Sin embargo, la incidencia se eleva en estudios poblacionales en mujeres de entre 20 y 32 años a alrededor de 100 casos nuevos/100.000 personas/año (Ghaderi y Scott, 2001). En la población general, la incidencia de BN parece ser mayor que la de la AN (Hoek y cols., 1995; Soundy y cols., 1995), situándose en cifras de 13 casos/100.000/año para todas las edades (Hoek, 2006) y elevándose hasta casi 500 casos/100.000/año en mujeres entre 20 y 32 años (Ghaderi y Scott, 2001).

En cuanto a la prevalencia con respecto al sexo, los TCA afectan mucho más a la mujer que al varón. Se han señalado cifras de prevalencia en varones de 0,2 y 0,3% de AN, 0,1 y 0,5% de BN y 1,1 y 3,1% de TCANE (Raevuori y cols., 2014) En cuanto a los ratios para todo el conjunto de trastornos, se sitúan entre 1:6 y 1:10 de varones frente a mujeres (García-Camba, 2001).

Los valores para la población española son heterogéneos, con tasas de prevalencia global de TCA (ambos sexos) que oscilan entre 2,91% y 3,7%. En pacientes femeninos la prevalencia de AN oscila entre 0,10% y 0,69%, la de BN entre 0,55% y 1,38% y en cuanto a las formas subclínicas (TCANE) la prevalencia oscila entre 2,10% y 4,86% (Morande y cols., 1999; Perez-Gaspar y cols., 2000; Rojo y cols., 2003; Beato-Fernandez y cols., 2004; Rodriguez-Cano y cols., 2005).

2.1.3. Etiopatogenia

Los TCA son enfermedades psiquiátricas complejas, caracterizadas por distintas alteraciones en el comportamiento alimentario y que se asocian con una importante comorbilidad psiquiátrica y fisiopatológica. Estos trastornos son enfermedades psicosomáticas graves de origen multifactorial. Los principales factores que intervienen en la etiopatogenia de los TCA son de cuatro tipos:

- *Biológicos*, fundamentalmente genéticos y endocrinos.
- *Psicológicos*, que incluyen los trastornos emocionales de la personalidad.
- Las alteraciones del *entorno familiar*.
- *Socioculturales*, principalmente en el mundo occidental donde la delgadez se relaciona con atractivo físico (García-Camba, 2001).

Desde esta perspectiva, el modelo etiopatogénico más aceptado en la actualidad es el *biopsicosocial*, siendo también el más ampliamente utilizado para el abordaje terapéutico y preventivo. El primer modelo conocido que trató de poner en evidencia todo este conjunto de factores, es el modelo multifactorial de la AN propuesto por Lucas (Lucas y cols., 1988). Dicho modelo parte de tres factores: la predisposición biológica, la predisposición psicológica y el entorno social, que interactuarían entre sí para dar lugar al TCA. De forma paralela, Toro y Vilardiell (Toro,

1987) consideran la existencia de una serie de factores predisponentes, precipitantes y mantenedores del trastorno (Tabla 1).

Factores predisponentes	Factores genéticos Edad (13-30 años) Sexo femenino Introversión/inestable Obesidad Nivel social del medio Familiares con trastornos afectivos o con adicciones Obesidad materna Valores estéticos dominante
Factores desencadenantes	Cambios corporales adolescentes Separaciones y pérdidas Rupturas conyugales de los padres Incremento rápido de peso Críticas con respecto al cuerpo Enfermedades adelgazantes Traumatismo desfigurador Incremento de la actividad física Acontecimientos vitales o hechos estresantes
Factores de mantenimiento	Consecuencias de la inanición Interacción familiar Aislamiento social Cogniciones anoréxicas Actividad física excesiva Yantrogenia (efecto de una mala intervención)

Tabla 1. Factores predisponentes, desencadenantes y de mantenimiento del trastorno anoréxico (adaptado (Toro, 1987)).

2.1.4. Clasificación

La clasificación actual de los TCA fue iniciada por la American Psychiatric Association (A.P.A.) en el DSM III (1983). En esta primera clasificación se establecieron cinco categorías diagnósticas: AN, BN, pica, rumiación, y TCANE. En

una posterior revisión del DSM III, los TCANE se mantuvieron bajo la denominación de trastornos de difícil diagnóstico o inusuales, recogidos en el concepto de categorías residuales. En 1994, la A.P.A. publicó el DSM IV (A.P.A., 1994), donde se recogen nuevos aspectos sobre la clasificación de los TCA:

- Los TCA aparecen como una entidad independiente, distinta a los trastornos relacionados con la infancia y la adolescencia.
- La pica y la rumiación no están recogidos.
- Dentro del epígrafe TCANE, se incluye un nuevo trastorno denominado *trastorno por atracón* (TA).

En este trabajo seguimos el DSM IV revisado (A.P.A., 2000) (Tabla 2).

Trastorno
ANOREXIA NERVIOSA (F50.0) • Tipo restrictivo • Tipo compulsivo-purgativo
BULIMIA NERVIOSA (F50.2) • Tipo purgativo • Tipo no purgativo
TRASTORNO DE LA CONDUCTA ALIMENTARIA NO ESPECIFICADO (F50.9) • Casos parciales de AN • Casos parciales de BN • Trastorno por atracón

Tabla 2. Clasificación de los trastornos de la conducta alimentaria según el DSM-IV-TR.

2.2. ANOREXIA NERVIOSA

2.2.1. Criterios diagnósticos

Podemos definir la AN como "*una restricción patológica y voluntaria del deseo de comer, rechazo a mantener el mínimo peso normal junto a un miedo intenso a ganar peso y una alteración en la percepción de la imagen corporal*" (Turón, 1997). Los criterios diagnósticos de AN de acuerdo al DSM IV-TR incluyen:

- Peso corporal por debajo del 85% recomendado para la edad y altura.
- Miedo intenso a ganar peso.
- Distorsión de la imagen de sí mismo.
- Amenorrea de larga duración (al menos tres meses).
- Severa restricción calórica y déficit de nutrientes y deshidratación (en estados avanzados) asociados con una seria comorbilidad.

De acuerdo con el DSM-IV, los pacientes pueden ser clasificados en dos subgrupos:

- Tipo restrictivo (ANR): alcanzan valores de peso corporal mínimos mediante dietas restrictivas drásticas, acompañadas de un intenso ejercicio físico.
- Tipo compulsivo/purgativo (ANCP): en este subtipo los pacientes pueden participar en atracones y purgas, sólo atracones (con períodos intermitentes de ayuno o de ejercicio excesivo), o sólo purgas (es decir, la práctica de vómito auto-inducido, uso de laxantes o de otras formas extremas de control de peso).

2.2.2. Clínica

La AN ocurre mayormente durante la adolescencia, aunque cada vez aparecen más casos en niñas prepuberales. Físicamente, los pacientes de AN presentan un aspecto delgado o excesivamente delgado [IMC (índice de masa corporal) < 17,5] y con frecuencia son de talla baja.

En cuanto al perfil psicológico de estos pacientes, presentan baja autoestima, inseguridad, rigidez y distorsiones cognitivas tales que consideran su valía personal en función de su silueta y de su capacidad para mantenerla y bajar el peso (Turón, 1997). El perfeccionismo, la obsesión y la rigurosidad son rasgos muy extendidos entre los pacientes, y son evidentes durante el desarrollo de la enfermedad y, a largo plazo, después de la recuperación (Halmi y cols., 2000). Los pacientes de AN presentan un amplio rango de alteraciones del estado afectivo y emocional. Son característicos un estado de ánimo general deprimido, el vacío emocional, el aislamiento social y la pérdida de la libido (Herpertz-Dahlmann, 2009). Asimismo, son frecuentes los desórdenes psiquiátricos como la depresión, la ansiedad, los trastornos obsesivo-compulsivos, el abuso de sustancias y otros trastornos de la personalidad (Pollice y cols., 1997).

La AN lleva asociada una importante comorbilidad no sólo psiquiátrica, también fisiopatológica, como consecuencia del estado de inanición mantenido. Las complicaciones cardiovasculares son frecuentes, descritas hasta en un 80% de los casos, fundamentalmente en forma de bradicardia, hipotensión, arritmias, alteraciones de la repolarización y muerte súbita (Vazquez y cols., 2003). En el caso de la ANCP, los trastornos electrolíticos pueden causar arritmias mortales (Rome, Ammerman et al. 2003). También existe un trastorno endocrino generalizado que afecta al eje hipotálamo-hipofisario-gonadal y somatomedínico manifestándose con amenorrea y osteoporosis, además de otras alteraciones hormonales. Si el inicio del trastorno es anterior a la pubertad o se produce durante su desarrollo, se retrasa la secuencia de las manifestaciones de la pubertad, o incluso se detiene (Martinez-Sopena, 2004). En cuanto a los datos bioquímicos y hematológicos se observa leucopenia, trombocitopenia, anemia, niveles bajos de glucosa, niveles bajos de electrolitos y aumento de colesterol (Herpertz-Dahlmann, 2009).

Es importante señalar que los pacientes con AN presentan un riesgo de muerte prematura tres veces mayor que el del resto de la población (Millar y cols., 2005). Las principales causas de mortalidad son la inanición, el suicidio y la muerte súbita de causa cardíaca (Sullivan, 1995).

2.2.3. Factores de riesgo

Anteriormente nos hemos referido a la AN como un trastorno complejo que depende de varios factores que pueden agruparse dentro un modelo biopsicosocial: factores biológicos (genéticos y neuroendocrinos), psicológicos, familiares y socioculturales (Lucas y cols., 1988). Una combinación desfavorable de estos factores contribuye al aumento de la susceptibilidad a desarrollar el trastorno (Sulek y cols., 2007).

Un factor que confiere un alto riesgo de padecer tanto AN como BN es la realización de manera asidua de dietas severas (Patton y cols., 1999). Otros factores tan diversos como pertenecer al sexo femenino, el tipo de alimentación en la infancia, problemas gastrointestinales, la baja autoestima, el haber sufrido abusos sexuales y otras experiencias adversas y la presencia de trastornos psiquiátricos como el trastorno obsesivo-compulsivo y la ansiedad, se han identificado como factores de riesgo (Bulik y cols., 1997; Jacobi y cols., 2004). Hoy en día, existe una importante influencia de los factores socio-culturales, en especial en el mundo occidental, donde la sobreabundancia de alimentos se identifica con bienestar social y la delgadez se considera sinónimo de atractivo físico (García-Camba, 2001). En posteriores apartados de esta introducción se describirá la literatura reciente que apunta a que la presencia de polimorfismos genéticos en las rutas de genes del sistema nerviosos central puede constituir también un factor de riesgo para la AN.

2.2.4. Diagnóstico diferencial

La distinción entre los diagnósticos de AN, BN y TCANE es difícil de hacer en la práctica debido a que existe un considerable solapamiento entre las características clínicas de las mismas. Asimismo, no es inusual que un paciente con un desorden alimenticio pase por varios diagnósticos conforme evoluciona su trastorno.

El DSM IV-TR establece la necesidad de tener en cuenta otras posibles causas de pérdida de peso, especialmente cuando hay un inicio muy tardío de la enfermedad. En esos casos, no suele presentarse una imagen distorsionada del cuerpo ni una ideación obsesiva de la silueta y/o la comida. En primer lugar, se debe realizar el diagnóstico diferencial con algunas enfermedades que se asocian con pérdida de peso como hipertiroidismo y otros trastornos endocrinológicos, enfermedades que cursan con malabsorción, vómitos, diarreas, etc. También existe solapamiento de síntomas con trastornos psiquiátricos como el trastorno depresivo, ansiedad o trastorno dismórfico corporal (Díez, 2010).

Para finalizar, el diagnóstico diferencial entre AN y BN se establece mediante la presencia de episodios de atracones y conductas compensatorias encaminadas a no ganar peso, típicas de pacientes con BN. Los pacientes con BN, al igual que los pacientes con ANCP, presentan excesiva preocupación por la apariencia física, pero la principal diferencia entre ambas entidades clínicas es que los pacientes con BN suelen presentar un peso dentro o por encima de los límites de normalidad en contraposición con los pacientes con ANCP que presentan un peso por debajo del peso normal mínimo.

2.3. BULIMIA NERVIOSA

2.3.1. Criterios diagnósticos

En 1979 surge el concepto de BN como una variante de la AN, y se establecen los primeros criterios diagnósticos (Russell, 1979). En la BN existen períodos de dieta y ayuno que son interrumpidos por episodios de atracones compulsivos acompañados por una sensación de pérdida de control. Los criterios diagnósticos de la BN de acuerdo al DSM IV-TR incluyen:

- Episodios de atracones recurrentes.
- Comportamientos compensatorios inapropiados
- Frecuencia de los atracones de al menos dos veces por semana durante un periodo mínimo de tres meses.
- La autoestima depende del aspecto y peso corporal.
- El trastorno no ocurre exclusivamente durante periodos de AN.

Durante los atracones se consume en exceso una gran cantidad de comida, situación que conduce al paciente a adoptar conductas compensatorias inapropiadas que eviten la ganancia de peso y que son diferentes en los dos subtipos de bulimia (García-Camba, 2001).

- *Tipo purgativo:* medidas compensatorias que incluyen el vómito auto-inducido, laxantes, diuréticos y cualquier otro tipo de medicamento.
- *Tipo no purgativo*: los episodios compulsivos se alternan con episodios de ayuno o de muy poca ingesta de alimentos y ejercicio físico muy intenso (Ballabriga y Carrascosa, 2001).

2.3.2. Clínica

Los pacientes con BN suelen ser mayores que los pacientes con AN y un cuarto de los mismos tienen una historia previa de AN. A diferencia de los pacientes anoréxicos, los pacientes bulímicos suelen presentar un apetito fuerte y un deseo manifiesto de comer con fluctuaciones de peso que están dentro o por encima de los niveles normales (Herpertz-Dahlmann, 2009) Por tanto, la ausencia de un patrón corporal típico, unido a la vergüenza que sienten por su comportamiento que les lleva a esconder o negar sus actos, hace más difícil el diagnóstico de BN (Myers, 2006).

En cuanto al perfil psicológico, las pacientes suelen presentar tendencia al perfeccionismo, necesidad de aprobación, egocentrismo, identidad lábil, distorsión de la realidad, etc. (Morande, 1999).

En relación a la comorbilidad psiquiátrica, las pacientes con BN presentan las mismas alteraciones psiquiátricas que se han citado anteriormente para pacientes con AN (depresión, ansiedad, trastorno obsesivo-compulsivo, abuso de sustancias, etc). Sin embargo, la incidencia de suicidio o intentos de suicidio es más alta en pacientes con BN que en pacientes con AN. Se han registrado cifras de entre 25 y 35% de pacientes con BN que presentan tentativa de suicidio en su historial clínico (Herpertz-Dahlmann, 2009).

Entre las manifestaciones fisiopatológicas, no es frecuente la aparición de amenorrea. Por otro lado, las pacientes son susceptibles de padecer alcalosis metabólica hipokalémica (en casos de purga severa) y acidosis metabólica (en casos de uso severo de laxantes). Son frecuentes las alteraciones derivadas de la provocación del vómito como erosiones en la dentadura y desgarros esofágicos, así como aquellas derivadas de la sobreingesta como la dilatación del estómago (Farrera Sabioncello, 2001).

2.3.3. Factores de riesgo

Entre los factores de riesgos más comunes de la BN encontramos:

- Factores biológicos: neuroendocrinos, predisposición a la obesidad, rasgos de personalidad, Diabetes Mellitus, etc.
- Psicológicos: antecedentes personales y familiares de enfermedades mentales, tendencia a la depresión y dependencia del alcohol o las drogas, separaciones, pérdidas de seres queridos, abusos y negligencias, abusos sexuales, traumas psicológicos, dietas extremas, distorsiones cognitivas.
- Sociales: psicopatología familiar, estructura familiar deteriorada, violencia en la familia, separción/divorcio o nuevo matrimonio, presión cultural y de los medios de comunicación.(French, 1999; Farrera Sabioncello, 2001)

Más adelante comentaremos varios estudios que sugieren la existencia de factores de riesgo genéticos en el desarrollo tanto de BN como de AN.

2.3.4. Diagnóstico diferencial

El diagnóstico diferencial de BN suele ser más complicado, debido a que las alteraciones físicas en un principio son menores y el paciente puede ocultar datos acerca de su comportamiento. Hay que tener en cuenta trastornos gastrointestinales que cursan con vómitos repetidos, alteraciones profundas de la personalidad que cursan con trastornos de la conducta alimentaria, dependencia al alcohol, conducta antisocial, trastornos depresivos e hiperfagias de causa orgánica neurológica (Síndrome de Kleine-Levin) (García-Camba, 2001).

Aunque el sistema de clasificación DSM no diferencia entre pacientes bulímicos con o sin antecedentes de AN debe tenerse en cuenta este factor, no sólo como antecedente del trastorno alimentario sino también en el caso de concurrencia. En tal caso, si los episodios bulímicos aparecieran exclusivamente durante la AN, se

diagnosticaría AN subtipo compulsivo-purgativo y no se establecería el diagnóstico adicional de BN. La BN se podría considerar, en algunos casos, como una secuela de la AN persistente, pudiéndose dar también la secuencia contraria (Giner-Lladós, 2011).

2.4. PREDISPOSICIÓN GENÉTICA A LOS TRASTORNOS DE LA CONDUCTA ALIMENTARIA

La predisposición genética en los TCA es un hecho actualmente aceptado. Existen numerosos estudios epidemiológicos realizados en familias que sugieren que los factores genéticos podrían influir notablemente en el desarrollo del trastorno alimentario. Los estudios realizados en gemelos monocigóticos y dicigóticos son una poderosa herramienta para diferenciar entre componentes ambientales y genéticos. De todos estos trabajos se deriva una heredabilidad de entre el 48% y el 88% para AN y de entre el 28% y el 83% para BN, (Bulik y cols., 1998; Kipman y cols., 1999; Bulik y cols., 2000; Gorwood y cols., 2003). Además, los rasgos psicológicos que presentan con frecuencia los pacientes con TCA, también muestran un componente hereditario. Se han analizado varias escalas del Inventario de Trastornos de la Conducta Alimentaria (del inglés *Eating Disorder Inventory*, EDI-2) a este respecto. Por ejemplo, la *Obsesión por la delgadez*, definida como una excesiva preocupación por el peso, las dietas y el miedo a engordar (Garner y cols., 1983) se ha analizado en estudios con gemelos y el componente hereditario se ha estimado entre un 45 y 50% (Rutherford y cols., 1993; Keski-Rahkonen y cols., 2005). Igualmente, *Perfeccionismo* también presenta contribuciones genéticas significativas (Kamakura y cols., 2003; Wade y cols., 2008).

Hasta la fecha, los factores de riesgo genético que subyacen en los TCA son, en gran parte, desconocidos. Como en la mayoría de las enfermedades psiquiátricas, la heredabilidad de los TCA parece seguir un patrón no mendeliano, lo que sugiere

que un gran número de genes, que abarcan varias regiones del genoma, pueden estar implicados en la susceptibilidad.

En los últimos años, los mayores esfuerzos se han encaminado a descifrar los mecanismos moleculares responsables del desarrollo de los TCA. En una revisión se recogen 175 estudios de asociación con posibles genes candidatos en pacientes con AN. Se identificaron un total de 128 polimorfismos localizados en 43 genes distintos que codificaban proteínas implicadas en rutas biológicas diversas (Rask-Andersen y cols., 2010). En la tabla 3, se muestra un resumen de las rutas que pueden estar implicadas en la patogenia de los TCA.

Rutas relacionadas con los TCA	Componente asociado a TCA
Sistemas de neurotransmisión relativos a la salud mental	Serotonina (5-HT) Factor neurotrófico derivado del cerebro (BDNF) Norepinefrina (NE) Glutamato (NMDA) Canal SK3 (KCCN3)
Sistemas de regulación de la ingesta	Leptina Proteina relacionada con Agouti (AGRP) Hormona estimulante de melanocito (MSH) Receptor de melanocortina 4 (MC4R) Neuropétido Y (NPY) Grelina Colecistoquinina (CCK).
Sistemas de alimentación y motivación relacionadas con la recompensa	Opiáceos Cannabinoides OPRD1 (anandamida (AEA), THC, CBR1) Dopamina (receptores DRD2, DRD3, DRD4) Catecol-O-metil-transferasa (COMT).
Sistemas de regulación del metabolismo de la energía	Proteínas desacoplantes 2 y 3 (UCP2 y UCP3)
Sistemas neuroendocrinos con énfasis en las hormonas sexuales	Receptor estrogénico β (ESR2)
Sistema inmune y la respuesta inflamatoria	Factor de necrosis tumoral alfa (TNF-α).

Tabla 3. Rutas implicadas en los estudios de asociación genética sobre TCA (adaptado de (Rask-Andersen y cols., 2010))

Gracias a los avances en tecnología, en los últimos años han proliferado los estudios de asociación del genoma completo (conocidos como *GWAS*, del inglés *Genome-Wide Association Studies*) que están aportando nueva información sobre la existencia de factores genéticos que puedan conferir susceptibilidad de desarrollar un TCA. Varios de estos estudios (Wang y cols., 2011; Wade y cols., 2013; Boraska y cols., 2014) han puesto de manifiesto nuevas regiones cromosómicas de susceptibilidad que pueden servir como punto de partida para una investigación más detallada siguiendo el modelo de genes candidatos.

2.5. GENES DE EXPRESIÓN CEREBRAL IMPLICADOS EN EL DESARROLLO NEURONAL Y HOMEOSTASIS ENERGÉTICA

Los genes que codifican proteínas expresadas en el sistema nervioso central (SNC) han sido propuestos como genes candidatos a estudio en el campo de los TCA en numerosas publicaciones. En 2003 Connan y cols. (Connan y cols., 2003) propusieron un modelo de neurodesarrollo para AN. En él señalan que la combinación de determinados genes implicados en el desarrollo y maduración del SNC, junto con factores de estrés ambientales presentes durante las primeras etapas del desarrollo del SNC, pueden causar una disfunción del eje hipotálamo-hipófisis-adrenal, predisponiendo al paciente a padecer AN a lo largo de su vida.

De entre todos los genes con expresión neuronal propuestos, son de especial interés los genes que intervienen, de manera directa o indirecta, en el mecanismo molecular de los sistemas de control de la ingesta de alimentos y del peso corporal a nivel hipotalámico (Monteleone y Maj, 2008; Rask-Andersen y cols., 2010; Clarke y cols., 2012). En la revisión citada anteriormente (Rask-Andersen y cols., 2010), se proponen una serie de genes candidatos a estudio, de los que no existían evidencias acerca de su relación con los TCA. Entre ellos destacan genes implicados en rutas de control del apetito o asociados con obesidad. Es factible por tanto pensar que

alteraciones en las vías implicadas en la modulación de los comportamientos alimentarios y del control de la ingesta, puedan contribuir a desarrollar patrones conductuales aberrantes que favorezcan la instauración de un TCA.

En este trabajo nos vamos a centrar en el estudio de las alteraciones genéticas en el gen *BDNF* (factor neurotrófico derivado del cerebro), el gen *MC4R* (receptor 4 de la melanocortina) y el gen *NEGR1* (regulador del crecimiento neuronal 1) y su posible influencia sobre dos aspectos principales: la susceptibilidad a los TCA y los parámetros clínicos (antropométricos pero especialmente psicológicos) que presentan los pacientes con TCA.

Los tres genes presentan dos características comunes: tienen expresión en el SNC y se les ha relacionado con la obesidad y los mecanismos de control de la ingesta (Lee y cols., 2012; Nakazato y cols., 2012; Valette y cols., 2013). En los últimos años, estudios GWAS realizados en población obesa, han señalado que los genes *NEGR1, BDNF* y *MCR4* pueden desempeñar un papel importante en el desarrollo de la obesidad (Thorleifsson y cols., 2009; Willer y cols., 2009; Speliotes y cols., 2010; Wheeler y cols., 2013). A partir de estos estudios, se han llevado a cabo trabajos de asociación en distintas poblaciones que confirman la relación entre varios polimorfismos de estos genes y un riesgo aumentado de padecer obesidad y/o presentar rasgos característicos de esta patología, como aumento del IMC y acumulación de grasa corporal entre otras. (Hotta, 2009; Poveda y cols., 2014)

El gen *BDNF* es el más estudiado de los tres en relación a los TCA. Es importante destacar su conexión con el gen *MC4R* en el control del balance energético. Xu y cols. (Xu y cols., 2003) sostienen que durante el proceso de señalización del MC4R se activaría la expresión del BDNF en el hipotálamo ventromedial. Estos datos, por tanto, apoyarían la hipótesis de que el BDNF es un efector importante en la vía de señalización del MC4R y conectaría a ambos genes en el proceso de control del balance energético.

2.6. FACTOR NEUROTRÓFICO DERIVADO DEL CEREBRO (BDNF)

2.6.1. Estructura, síntesis y función

El BDNF es un miembro de la familia de proteínas denominadas neurotrofinas, las cuales presentan una estructura altamente conservada. La familia de las neurotrofinas incluye además del BDNF, el factor de crecimiento nervioso, la neurotrofina 3, la neurotrofina 4/5, y la neurotrofina 6 (Noble y cols., 2011).

El gen que codifica la proteína BDNF se localiza en el cromosoma 11p13 y está formado por 11 exones. Es sintetizado inicialmente como una proteína precursora, el preproBDNF, la cual es procesada proteolíticamente dando lugar al proBDNF y a continuación, a la proteína madura o BDNF. El proBDNF se une, de manera preferente, al receptor $p75^{NTR}$ y el BDNF al receptor tropomiosin kinasa tipo B (TrkB) (Nakazato y cols., 2012) (Figura 1). Una vez que el ligando se ha unido, los receptores TrkB se dimerizan y se desencadena la activación catalítica, que resulta en la autofosforilación del receptor y en la posterior activación de una cascada de señalización (Hennigan y cols., 2007).

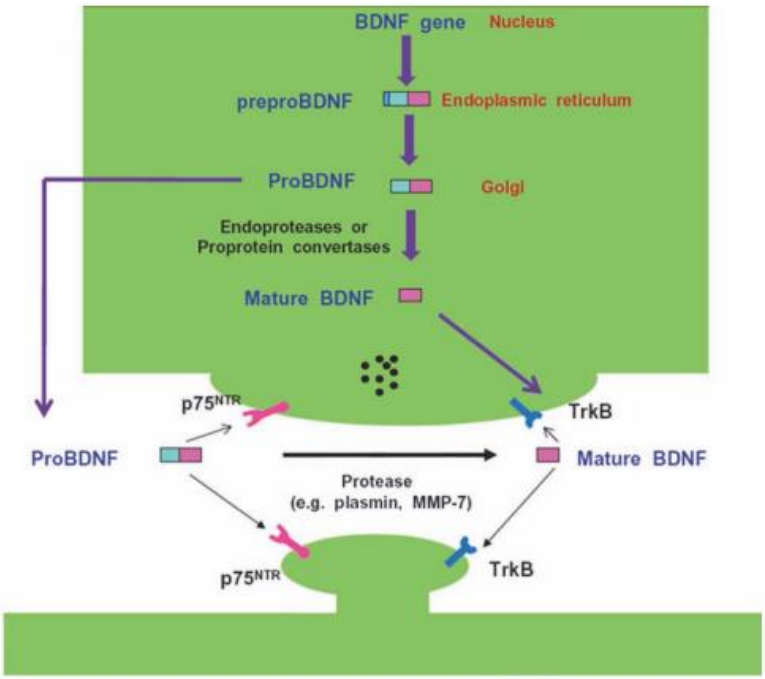

Figura 1. Síntesis BDNF (Hashimoto, 2010).

El *BDNF* juega un papel clave en las etapas tempranas de la embriogénesis, potenciando la diferenciación de las células madres precursoras del sistema nervioso central. En etapas tardías del desarrollo, el *BDNF* regula el crecimiento dendrítico cortical, la ramificación y remodelación del nervio óptico, el desarrollo de las conexiones dopaminérgicas y de las neuronas del hipocampo.(Kernie y cols., 2000). También proporciona soporte trófico a las neuronas noradrenérgicas, dopaminérgicas y sertoninérgicas entre otras, cuyos neurotransmisores se encuentran alterados en muchas enfermedades mentales (Arija y cols., 2010).

Las neurotrofinas se han asociado con una amplia variedad de desórdenes neurodegenerativos y han sido consideradas como estrategias terapéuticas. Además, los altos niveles de expresión de BDNF y de su receptor TrkB en la región cortical y en el hipocampo, junto con su función crítica en el mantenimiento de las conexiones sinápticas, plasticidad sináptica y neurotransmisión, han favorecido su asociación con la regulación de los procesos cognitivos como el aprendizaje y la memoria (Bath y Lee, 2006).

Varios trabajos destacan el importante papel que desempeña el gen *BDNF* en el control del balance energético y en los centros de recompensa del cerebro, lo que repercutiría en el comportamiento alimentario, el peso corporal y el deterioros cognitivos (Kernie y cols., 2000; Gray y cols., 2006; Burns y cols., 2010; Brandys y cols., 2011; Cordeira y Rios, 2011; Rosas-Vargas y cols., 2011). En estudios realizados con ratones knockout (Kernie y cols., 2000; Fox y Byerly, 2004) o con deleción condicional (supresión de la expresión del *BDNF* del cerebro tras el nacimiento) (Rios y cols., 2001) se ha observado que los animales manifiestan un comportamiento hiperfágico, hiperactivo y ansioso, además de ganancia de peso.

En relación a las concentraciones séricas de BDNF, existen varias publicaciones que constatan la existencia de valores disminuidos en pacientes con BN y AN en comparación con población control (Nakazato y cols., 2003; Monteleone y

cols., 2004). Por otro lado, se han obtenido correlaciones negativas entre los niveles séricos de BDNF y las puntuaciones obtenidas en cuestionarios psicopatológicos (Mercader y cols., 2007; Mercader y cols., 2010). Estos datos sugieren que los niveles de BDNF en suero pueden encontrarse alterados en pacientes con TCA y modular la psicopatología asociada a estos trastornos.

2.6.2. Polimorfismos genéticos del gen BDNF

En los últimos años se han descrito varios polimorfismos genéticos del gen *BDNF*. Los dos polimorfismos más estudiados han sido el rs6265 (Val66Met) y el rs56164415 (C-270T) que se han asociado principalmente con tres tipos de patologías, aunque con resultados contradictorios:

- Enfermedades psiquiátricas como la esquizofrenia (Chen y cols., 2006; Jonsson y cols., 2006; Zhang y cols., 2006), el trastorno bipolar (Schumacher y cols., 2005; Green y cols., 2006), la depresión (Schumacher y cols., 2005; Lee y cols., 2014), los TCA (Ribases y cols., 2003; Ribases y cols., 2004; Brandys y cols., 2013), la ansiedad (Jiang y cols., 2005) y el abuso de sustancias (Cheng y cols., 2005; Itoh y cols., 2005; Zhang y cols., 2006).
- Trastornos neurodegenerativos como la enfermedad de Parkinson y Alzheimer.(Ventriglia y cols., 2002; Bian y cols., 2005; Nishimura y cols., 2005; Saarela y cols., 2006).
- Obesidad (Friedel y cols., 2005; Beckers y cols., 2008; Skledar y cols., 2012).

2.6.2.1. Polimorfismo rs6265

El polimorfismo rs6265 también conocido como Val66Met o 196G/A, consiste en la sustitución de una valina por una metionina en el codón 66 del prodominio de la proteína (Figura 2), y aunque no afecta a la función de la proteína madura, se ha visto que altera el transporte intracelular y el empaquetamiento del proBDNF y como consecuencia afecta a la secreción de la proteína madura (Egan y cols., 2003;

Hashimoto, 2010). Fue el primer polimorfismo del gen *BDNF* estudiado y parece tener una gran influencia en los procesos cognitivos en humanos.

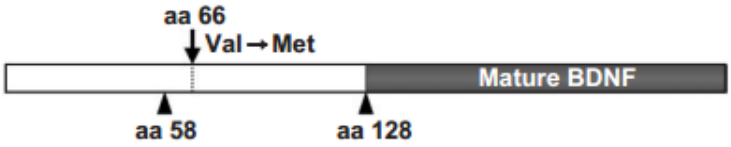

Figura 2. Estructura proBDNF. Se muestran los sitos de corte de dos proteasas implicadas en el procesamiento de la proteína y la posición del polimorfismo rs6265. (Hashimoto y cols., 2005)

Un estudio epidemiológico concluye que entre el 30-50% de la población mundial es heterocigota (Val/Met) u homocigota (Met/Met) para el polimorfismo (Shimizu y cols., 2004), aunque estos porcentajes presentan alta variabilidad geográfica y étnica. Es difícil realizar estudios en poblaciones de homocigotos del gen recesivo (Met/Met) debido a la baja frecuencia, que se ha estimado en torno al 4% en la población estadounidense (Shimizu y cols., 2004).

Con el objetivo de elucidar el mecanismo molecular alterado, se han realizado varios trabajos en cultivos celulares *in vitro*. En resumen, estos estudios parecen indicar que existe una señal en el prodominio de la región génica del *BDNF,* que incluye la sustitución de valina por metionina, la cual regula el tráfico intracelular de la proteína BDNF en la ruta secretora y que es necesaria para una salida eficiente de la misma (Bath y Lee, 2006). Recientemente, se ha identificado una proteína, la sortilina, que parece ser necesaria en el proceso de salida del BDNF en la vía secretora. Esta proteína interactúa específicamente con la región que incluye el polimorfismo y parece ser que el cambio Val/Met en la secuencia proteica, altera la interacción sortilina-BDNF (Chen y cols., 2005).

El polimorfismo rs6265 se ha asociado con episodios de deterioro mental y con reducción del volumen del hipocampo (Egan y cols., 2003; Bueller y cols., 2006). También se ha relacionado con mayor susceptibilidad de padecer trastornos

neuropsiquiátricos como el Alzheimer (Ventriglia y cols., 2002), la enfermedad de Parkinson (Momose y cols., 2002), la depresión (Sen y cols., 2003), TCA (Ribases y cols., 2003; Ribases y cols., 2004), y el trastorno bipolar (Neves-Pereira y cols., 2002). Además, varios trabajos han relacionado el polimorfismo con obesidad (Beckers y cols., 2008; Skledar y cols., 2012).

En los últimos años, se ha intentado determinar la posible influencia del polimorfismo rs6252 en el riesgo de padecer TCA. Los trabajos realizados ofrecen resultados contradictorios (Tabla 4). Sin embargo, un meta-análisis que incluyó 9 de estos estudios, concluye que la variante Val66Met no está asociada con AN significativamente (Brandys y cols., 2013). La principal limitación de este meta-análisis es que no analiza el efecto del polimorfismo con los subtipos de AN (restrictivo/purgativo) de manera independiente y por tanto, los resultados obtenidos podrían enmascarar la asociación de la variante con tan solo uno de los subtipos de la enfermedad.

Por otra parte, se han realizado estudios en sujetos sanos que establecen una relación entre variantes alélicas del gen *BDNF* y determinados rasgos de la personalidad como el neuroticismo (Sen y cols., 2003), la introversión (Terracciano y cols., 2010) y varios patrones relacionados con ansiedad y depresión (Lang y cols., 2005). Algunos síntomas de la ansiedad y la depresión se han asociado a su vez con los TCA (Godart y cols., 2002; Godart y cols., 2007), sin embargo, se han realizado pocos estudios en pacientes con TCA que pongan de manifiesto la posible influencia de las variantes genéticas del gen *BDNF* en los rasgos de la personalidad y síntomas psicopatológicos.

REFERENCIA	N	PRINCIPALES HALLAZGOS
(Ribases y cols., 2003)	ANR=26, ANCP=36, BN=70, Controles=112	Asociación alelo Met con riesgo de ANR. Valores más bajos de IMC en portadores alelo Met
(Ribases y cols., 2004)	AN=753, BN=389, Controles=510	Asociación alelo Met con riesgo todos los subtipos
(Koizumi y cols., 2004)	AN=97, BN=101, Controles=222	Asociación global con riesgo TCA y en particular, con riesgo BN.
(Friedel y cols., 2005)	AN=118, BN=80, Controles=96	No asociación con riesgo TCA
(Rybakowski y cols., 2007)	AN=149, Controles=100	No asociación con riesgo AN ni con dimensiones de la personalidad.
(Ando y cols., 2012)	AN=689; Controles= 573	No asociación con riesgo AN. Asociación alelo Met con valores más bajos en escala *Evitación del Daño.*
(Brandys y cols., 2013)	AN=235, Controles=643	No asociación con riesgo AN
(de Krom y cols., 2005)	AN=195, Controles=580	No asociación con riesgo AN
(Yilmaz y cols., 2014)	AN=745, BN=245, Controles=321	No asociación con riesgo de AN, BN o IMC

Tabla 4. Estudios de asociación relacionados con el polimorfismo *rs6252* en el gen BDNF en pacientes con trastornos de la conducta alimentaria.
AN: Anorexia Nerviosa; ANR: Anorexia Nerviosa Restrictiva; ANCP: Anorexia Nerviosa Purgativa; BN: Bulimia Nerviosa, IMC: índice de masa corporal; Met, metionina

2.6.2.3. Polimorfismo C-270T

El polimorfismo C-270T (rs56164415) fue descrito en 2001 como un SNP situado en el exón V de la región promotora no codificante del gen *BDNF* asociado con la enfermedad de Alzheimer (Kunugi y cols., 2001). Consiste en un cambio de una citosina por una timina en la posición 270 y aunque se desconoce el mecanismo molecular en el que está implicado, se ha postulado que este cambio puede influir en la transcripción del gen *BDNF* y afectar así a la expresión de la proteína (Zhai y cols., 2012).

Se han llevado a cabo numerosos estudios de asociación del polimorfismo con diferentes trastornos psiquiátricos con el fin de establecer si confiere susceptibilidad, tanto para el desarrollo de la enfermedad, como para los distintos síntomas característicos de las mismas. Existen numerosos trabajos de asociación del polimorfismo con la esquizofrenia con resultados, en la mayoría de los casos, discordantes (Szekeres y cols., 2003; Anttila y cols., 2005; Galderisi y cols., 2005; Watanabe y cols., 2006; Zhai y cols., 2012). Un meta-análisis reciente concluye que existe asociación del alelo T con esta patología en población del este asiático, pero no en población caucásica (Watanabe y cols., 2013) y que esta diferencia puede explicarse por la heterogeneidad genética entre ambas poblaciones. Otras patologías psiquiátricas como el abuso de sustancias o los ataques de pánico también han sido objeto de estudio en trabajos de asociación (Itoh y cols., 2005; Shimizu y cols., 2005).

Del mismo modo, se ha estudiado la influencia del polimorfismo en las enfermedades neurodegenerativas como el Alzheimer, el Parkinson o la Enfermedad de Huntington (Kunugi y cols., 2001; Nishimura y cols., 2005; Kishikawa y cols., 2006; Saarela y cols., 2006). La discrepancia entre los resultados obtenidos en estos trabajos puede estar influenciada por el distinto origen de las poblaciones de estudio, por lo que sería necesaria la realización de trabajos de asociación en poblaciones multiétnicas.

Al igual que en el caso del polimorfismo rs6265, el polimorfismo C-270T ha sido objeto de estudio en trabajos de asociación con patologías de la conducta alimentaria, aunque en menor número que el anterior. Todos los trabajos que se han realizado hasta el momento concluyen que no existe asociación entre el polimorfismo y el riesgo de padecer un TCA (Ribases y cols., 2003; Ribases y cols., 2004; de Krom y cols., 2005; Friedel y cols., 2005; Rybakowski y cols., 2007). Sin embargo, en relación a los parámetros psicopatológicos, el alelo mutante se ha asociado con valores más altos en la escala *Evitación del Daño* del Cuestionario *Temperamento y Carácter* (Rybakowski y cols., 2007). En la Tabla 5 se resumen los aspectos más importantes de estos estudios.

REFERENCIA	N	PRINCIPALES HALLAZGOS
(Ribases y cols., 2003)	ANR=26, ANCP=36, BN=70, Controles=112	No asociación con riesgo TCA
(Ribases y cols., 2004)	AN=753, BN=389, Controles= 510	No asociación con riesgo TCA. Asociación alelo C con inicio más tardío de pérdida de peso en pacientes BN
(Friedel y cols., 2005)	AN=118, BN=80,Controles=96	No asociación con riesgo TCA
(Rybakowski y cols., 2007)	AN=149, Controles=100	No asociación con riesgo AN. Asociación alelo T con valores elevados en escala de *Evitación del Daño* del *Cuestionario Temperamento y Carácter*
(de Krom y cols., 2005)	AN=195, Controles=580	No asociación con riesgo AN

Tabla 5. Estudios de asociación relacionados con el polimorfismo *C-270T* en el gen *BDNF* en pacientes con trastornos de la conducta alimentaria. AN: Anorexia Nerviosa; ANR: Anorexia Nerviosa Restrictiva; ANCP: Anorexia Nerviosa Purgativa; BN: Bulimia Nerviosa, EDI-2: Eating Disorders Inventory-2

2.7. RECEPTOR 4 DE LA MELANOCORTINA (MC4R)

2.7.1 Estructura, síntesis y función

Los receptores de melacortinas están codificados por una familia de genes que consta de cinco miembros: *MC1R, MC2R, MC3R, MC4R* y *MC5R*. Estos receptores unen a cuatro ligandos: α- β- y Ɣ-MSH (del inglés, *melanocyte-stimulating hormone,* hormona estimulante de melanocito) y a la hormona adrenocorticotropa (ACTH) (Switonski y cols., 2013).

El gen *MC4R* se encuentra situado en el cromosoma 18q21.3, está formado por un solo exón y codifica para una proteína de 332 aminoácidos (Gantz y cols., 1993). La expresión de los distintos receptores es tejido específica, siendo la expresión del *MC4R* principalmente cerebral, en regiones del tálamo, hipotálamo y el hipocampo, involucradas en funciones autónomas y endocrinas (Valette y cols., 2013).

El MC4R es un receptor con siete dominios transmembrana acoplado a la proteína G, que presenta un papel central en el sistema leptina-melanocortina y como consecuencia en la homeostasis energética (Hinney, 2013). El sistema leptina-melanocortina es activado inicialmente por la hormona leptina producida por el tejido adiposo. La leptina cruza la barrera hematoencefálica y se une a sus receptores en el núcleo arcuato. (Valette y cols., 2013). En el núcleo arcuato existen dos tipos de neuronas: las neuronas POMC/CART, las cuales trasfieren señales anorexigénicas a través de los derivados de la proopiomelacortina (POMC), y las neuronas AGRP/NPY, que transfieren señales estimuladoras de la ingesta a través del neuropéptido Y (NPY) y de la proteína relacionada con Agouti (AGRP). La leptina activa la síntesis de POMC, que por procesamiento proteolítico da lugar al α-MSH, el cual se une al MC4R produciendo inhibición de la ingesta. Por otro lado, la leptina inhibe las neuronas AGRP/NPY y suprime la expresión de AGRP, una proteína orexigénica que actúa como antagonista del MC4R (Robertson y cols., 2008). Por tanto, el MC4R integra tanto señales anorexigénicas proporcionadas por α-MSH, como señales orexigénicas

a través de su unión con ARPG, que dan como resultado la activación de mecanismos de saciedad o hambre, dependiendo de la señal dominante (Figura 3).

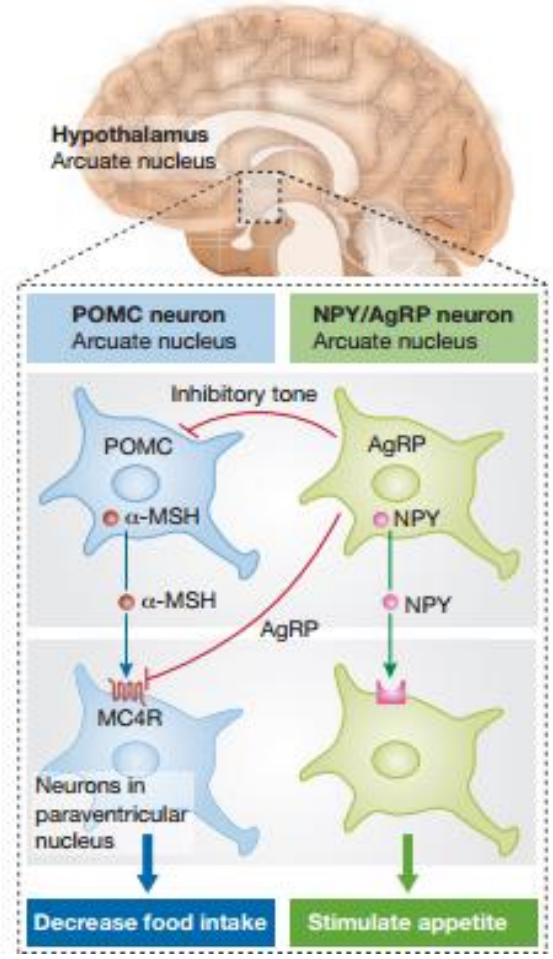

Figura 3: Esquema del efecto de α-MSH y AGRP sobre el receptor MC4R en la regulación de la ingesta. AgRP (del inglés, Agouti-related peptide, proteína relacionada con agouti); MC4R (del inglés, melanocortin 4 receptor, receptor 4 de la melacortina); α-MSH (del inglés, α-melanocyte stimulating hormone, hormona estimulante de melanocitos α); NPY (del inglés, neuropeptide Y, neuropétido Y); POMC (del inglés, proopiomelanocortin, proopiomelanocortina) (Rubinsztein, 2012).

Basándonos en el mecanismo molecular del MC4R, podríamos pensar que mutaciones en el gen producirían alteraciones en el sistema de señalización leptina-melanocortina por afectación de la etapa clave mediada por este receptor, que derivarían en patrones de comportamiento alimentario inadecuados con repercusión en el peso corporal.

En 1997, se publicaron los primeros estudios llevados a cabo en ratones *knockout* del gen *MC4R* (Huszar y cols., 1997) que concluían que los ratones que carecían de expresión del receptor presentaban hiperfagia, hiperinsulinemia, obesidad extrema y crecimiento lineal incrementado. Sin embargo, los ratones heterocigotos, con un alelo nulo de *MC4R* y un alelo normal, presentaban una ganancia de peso intermedia entre el fenotipo homocigoto mutante y el fenotipo homocigoto normal. Estos datos sugieren la existencia de una forma codominante de obesidad, con expresión fenotípica intermedia en el caso de sujetos heterocigotos.

2.7.2. Polimorfismos genéticos del gen *MC4R*

El gen *MC4R,* por su papel en el sistema de regulación de la ingesta*,* ha sido considerado principalmente como un gen relacionado con la obesidad. En 1998, se identificaron por primera vez, dos mutaciones que producían un desplazamiento en el marco de lectura del gen asociadas con esta patología. En el primer caso, Yeo y cols. (Yeo y cols., 1998) describieron una mutación consistente en una deleción de 4 pares de bases que generaba un codón de parada prematuro con síntesis truncada de la proteína MC4R. El paciente portador de la mutación presentaba comportamiento hiperfágico y búsqueda constante de comida. El segundo caso (Vaisse y cols., 1998), consistía en una inserción del mismo tamaño que la anterior, detectada en un paciente con obesidad de inicio precoz e hiperfagia. Dentro de la familia de este paciente todos los portadores de la mutación presentaban obesidad, y por el contrario, los no portadores presentaban un peso normal (Vaisse y cols., 1998).

Hasta el momento actual, se han identificado más de 160 variantes, principalmente en individuos obesos, que incluyen mutaciones no sinónimas, de desplazamiento del marco de lectura, sin sentido y deleciones (Hinney y cols., 1999; Hinney y cols., 2003; Farooqi y O'Rahilly, 2005; Lubrano-Berthelier y cols., 2006). La frecuencia con la que aparecen estas mutaciones en el gen *MC4R* en individuos con

obesidad mórbida es superior al 4% (Vaisse y cols., 2000; Farooqi, 2006) y normalmente se encuentran en heterocigosis.

Las variantes del gen se han clasificado en cinco grupos en función de sus efectos fenotípicos (Tao y Segaloff, 2005):

- Clase I: mutaciones que causan una síntesis defectuosa de la proteína o un aumento en su degradación, y por tanto, provocan como resultado una marcada disminución en su expresión.
- Clase II: mutaciones que causan retención del receptor dentro de la célula, probablemente, debido a un mal plegamiento de la proteína.
- Clase III: mutaciones que implican expresión de receptores en la superficie celular con baja afinidad por el ligando o con baja capacidad de unión al mismo.
- Clase IV: variantes que presentan alteraciones en el proceso de señalización.
- Clase V: variantes con efecto desconocido.

El polimorfismo rs17782313, localizado 150-188 kb después del gen *MC4R*, se ha identificado en estudios GWAS como un SNP con influencia en el IMC y en la circunferencia de la cintura y como un factor de riesgo de obesidad grave (Chambers y cols., 2008; Loos y cols., 2008). También se ha relacionado el alelo rs17782313C con fenotipos relacionados con el apetito, como el *picoteo*, en obesos de distintas poblaciones europeas y en europeos seleccionados al azar, tanto niños como adultos (Stutzmann y cols., 2009; Valette y cols., 2013). Recientemente, se ha asociado el polimorfismo rs17782313 con incrementos significativos en el volumen de materia gris en la amígdala derecha (región implicada en el comportamiento alimentario), y en el hipocampo derecho (estructura crucial en el proceso de memoria y aprendizaje) en mujeres homocigotas del alelo de riesgo. En el mismo trabajo se concluye que las mujeres portadoras del alelo de riesgo presentaban incrementos en las puntuaciones de *Desinhibición*, más concretamente en *Alimentación emocional,* del Cuestionario *Tres Factores de Alimentación* (Horstmann y cols., 2013).

Aunque la mayoría de las mutaciones en el gen *MC4R* se han asociado con obesidad, se han descrito dos SNP, que producen un cambio de aminoácido (Val103Ile y Ile251Leu), asociados con valores reducidos de peso corporal (Hinney, 2013). En el caso del SNP Val103Ile, se ha especulado que el efecto protector frente a obesidad puede ser debido a un incremento en la función del MC4R (Xiang y cols., 2006).

En relación a los TCA, existen pocos estudios publicados que pongan de manifiesto una asociación entre polimorfismos del gen *MC4R* y mayor riesgo de padecer estos trastornos, aunque cualquier gen implicado en el control del peso corporal podría considerarse un gen candidato para este tipo de patologías. Además, la obesidad se considera un factor de riesgo implicado en el desarrollo de la BN (Fairburn y cols., 1997), y por tanto sería factible pensar que las alteraciones genéticas que predisponen a padecer obesidad fueran más frecuente en la población bulímica que en una población control. Por otro lado, el TA ha sido considerado por algunos estudios como el principal fenotipo en portadores de mutaciones del gen *MC4R*, mientras que otros trabajos concluyen que no existe tal asociación (Tabla 6). La falta de uniformidad en los criterios diagnósticos utilizados para la evaluación del TA en las diferentes poblaciones de estudio puede contribuir a la disparidad de resultados (Valette y cols., 2013). Como hemos descrito anteriormente, también se han establecido asociaciones entre polimorfismos del gen con distintos patrones de comportamiento alimentario en población obesa que podrían hacerse extensible a la población afecta de TCA.

En la tabla 6 se resumen los estudios llevados a cabo en pacientes con TCA y varios estudios de asociación entre variantes del gen *MC4R* e ingesta por atracón en pacientes obesos.

REFERENCIA	SNP	N, TRASTORNO	PRINCIPALES HALLAZGOS
(Hebebrand y cols., 2002)	Tyr35Stop; Asp37Val	BN=81	Una única paciente con OB y BN presentó dos mutaciones funcionalmente relevantes.
(Brandys y cols., 2010)	rs17700633; rs17782313	AN=267, Controles=1636	No asociación con riesgo AN o con IMC
(Yilmaz y cols., 2014)	rs17782313	AN=745, BN=245, Controles=321	No asociación con riesgo AN, BN e IMC
(Branson y cols., 2003)	Cribado mutaciones por secuenciación.	OB=469, Controles=25	Todos los pacientes portadores de mutaciones presentaban comportamiento de IA
(Hebebrand y cols., 2004)	Cribado por SSCP	OB=1041	No asociación entre mutaciones en *MC4R* e IA después de descartar los polimorfismos no funcionales.
(Potoczna y cols., 2004)	Cribado mutaciones por secuenciación.	OB= 300	Todos los pacientes portadores de mutaciones presentaban comportamiento de IA.
(Lubrano-Berthelier y cols., 2006)	Cribado mutaciones por secuenciación.	OB=769, Controles=444	Los obesos portadores de mutaciones no presentaban IA ni ningún otro fenotipo característico
(Valette y cols., 2013)	Cribado mutaciones por secuenciación.	OB=4653	Mutaciones funcionales no se asociaron con IA
(Stutzmann y cols., 2009)	rs1782313	OB=2383, Controles=10489	Asociación del alelo C con alta incidencia de picoteo, mayor puntación en escala de hambre de *Stunkard* y alta prevalencia de ingesta de grandes cantidades de comida
(Horstmann y cols., 2013)	rs17782313	105 mujeres sanas, 116 varones sanos	Homocigotas alelo C presentaban incremento del volumen de materia gris en amígdala derecha y mayores puntaciones en desinhibición, más concretamente en alimentación emocional

Tabla 6. Estudios de asociación relacionados con mutaciones en el gen *MC4R* en pacientes con trastornos de la conducta y pacientes obesos con comportamiento de ingesta por atracón. AN: Anorexia Nerviosa, BN: Bulimia Nerviosa, OB: Obesidad, IA: Ingesta por atracón.

2.8. REGULADOR DEL CRECIMIENTO NEURONAL 1 (NEGR1)

2.8.1 Estructura, síntesis y función

El gen *NEGR1*, también conocido como Neurotractina (Marg y cols., 1999) o Kilon (del inglés *Kindred of IgLON*) (Funatsu y cols., 1999), codifica para una molécula de adhesión celular de la superfamilia de las inmunoglobulinas, perteneciente al subgrupo IgLON (Marg y cols., 1999). En mamíferos, el subgrupo IgLON incluye, además del gen *NEGR1*; a la neurotrimina (Ntm), la molécula de adhesión celular de unión a opioides (OBCAM), y la proteína de membrana asociada al sistema límbico (LAMP) (Speakman, 2013). Estas proteínas contienen tres dominios inmunoglobulina tipo C2 y se insertan en la membrana mediante un anclaje GPI (glicofosfatidilinositol) que posee de 6 a 7 sitios de glicosilación potenciales (Itoh y cols., 2008). Las proteínas de la familia IgLON se expresan en tejidos cerebrales y desempeñan un papel importante en el reconocimiento celular y el crecimiento de las neuritas a través de interacciones homofílicas y heterofílicas (Reed y cols., 2004).

Hasta la fecha, la función específica del gen *NEGR1 in vivo* es desconocida, aunque experimentos con ratones y ratas, indican un posible papel en el control neuronal del peso corporal y la ingesta (Lee y cols., 2012; Boender y cols., 2014). En este contexto, el gen *NEGR1* ha sido identificado en numerosos estudios GWAS como un locus asociado con obesidad (Thorleifsson y cols., 2009; Willer y cols., 2009; Speliotes y cols., 2010; Magi y cols., 2013; Wheeler y cols., 2013) y posteriores trabajos en diferentes cohortes han confirmado esta asociación (Renstrom y cols., 2009; Zhao y cols., 2009; Mejia-Benitez y cols., 2013).

El *NEGR1*, debido a su papel en el desarrollo neuronal, es además, un buen candidato a estudio en patologías psiquiátricas. En este campo, se ha asociado con la dislexia (Veerappa y cols., 2013) y se ha detectado un incremento de la expresión del mismo en ratas con administración crónica de Venlafaxina (un antidepresivo) (Tamasi

y cols., 2014). Recientemente, Maccarrone y cols. encontraron diferencias significativas en los niveles de proteína NEGR1 en el líquido cefalorraquídeo entre sujetos sanos y pacientes con depresión y trastorno bipolar.(Maccarrone y cols., 2013).

Por otro lado, un estudio reciente relaciona el gen *NEGR1* con el cáncer. Los autores observaron que la expresión del gen estaba disminuida en varios tejidos de cánceres humanos y que su papel en el reconocimiento e interacción celular puede ser clave en el control del crecimiento y transformación maligna (Kim y cols., 2014).

2.8.2. Polimorfismos genéticos del gen *NEGR1*

La mayoría de los SNPs del gen *NEGR1* descritos hasta el momento, se han investigado en los estudios GWAS referentes a obesidad citados en el anterior apartado. Además, un estudio reciente ha asociado dos variantes del gen *NEGR1* entre sí. Wheeler y cols. (Wheeler y cols., 2013) determinaron que la deleción de 43 kb (rs3101336) que confería mayor riesgo de obesidad estaba dirigida por la presencia de otra deleción de 8kb (rs1993709) situada en una región cercana en el genoma. Esta región de 8 kb, además, comprende el sitio de unión del factor de transcripción NKX6.1, que también participa en el desarrollo neuronal (Hafler y cols., 2008).

Por otro lado, Dennis y cols. (Dennis y cols., 2014) investigaron posibles efectos de SNPs de genes asociados con obesidad en la materia blanca cerebral de individuos sanos. Los investigadores determinaron que la variabilidad en el locus del gen *NEGR1*, y particularmente el alelo rs2815752A, estaba relacionado con una menor integridad de la materia blanca en una porción importante del cerebro, la cual se ha asociado con varias enfermedades psiquiátricas y neurodegenerativas (Ciccarelli y cols., 2008). Como el estudio se realizó en individuos sanos, los autores proponían que el gen *NEGR1* podría afectar la estructura cerebral independientemente de su influencia en la obesidad.

Como hemos mencionado anteriormente, las alteraciones en genes que predisponen a obesidad podrían contribuir a la instauración de conductas alimentarias que favorezca el desarrollo de un TCA. Por tanto, cualquier gen implicado en el control del peso corporal o ingesta alimentaria puede considerarse un gen candidato para este tipo de patologías. Sin embargo, hasta la fecha, y aparte del presente trabajo, sólo se ha llevado a cabo un estudio con el objetivo de determinar el efecto de variantes del gen *NEGR1* (SNP rs2568958) en el riesgo de padecer AN, siendo los resultados obtenidos a este respecto negativos (Brandys y cols., 2010).

3. OBJETIVOS

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Con las premisas anteriores, que sugieren que ciertos genes que codifican proteínas expresadas en el SNC son buenos candidatos a estudio en el campo de los TCA, y que ponen de manifiesto la relación entre la obesidad y los TCA, planteamos la siguiente hipótesis de trabajo:

La presencia de alteraciones genéticas en las rutas centrales implicadas en la regulación de la conducta alimentaria que se han asociado con la obesidad o el IMC también podrían jugar un papel relevante en (i) el riesgo a desarrollar AN o BN y/o (ii) en la aparición o la gravedad de síntomas psicopatológicos característicos. En este trabajo nos centraremos en el estudio de alteraciones genéticas en el gen *BDNF*, el gen *MC4R* y el gen *NEGR1*.

Los objetivos específicos de esta tesis son:

1. Selección y purificación de ADN genómico de controles sanos y pacientes de AN o BN.
2. Caracterización clínica-psicológica de las pacientes de TCA mediante entrevistas estandarizadas.
3. Análisis de 21 tag-SNPs del gen *NEGR1*, 4 tag-SNPs y dos polimorfismos adicionales (Val66Met y C-270T) del gen *BDNF* en el ADN de pacientes con AN, BN y controles sanos.
4. Estudio de la variabilidad del gen *MC4R* mediante secuenciación completa en sujetos obesos, pacientes con AN y pacientes con comportamiento de purgas y atracón diagnosticados de BN o de TA.
5. Estudio comparativo de las frecuencias alélicas, genotípicas y haplotípicas en los grupos mencionados anteriormente
6. Estudio del impacto de las variantes genéticas consideradas sobre parámetros antropométricos y psicológicos de las pacientes.

4. MATERIAL Y MÉTODOS

4.1. SELECCIÓN DE PARTICIPANTES EN EL ESTUDIO

4.1.1. Selección de pacientes

El grupo de estudio de pacientes para los genes *NEGR1* y *BDNF* consistió en 169 mujeres con diagnóstico de TCA. De estas, 106 presentaban AN (78 ANR y 27 ANCP) y 63 BN.

Para el estudio del gen *MC4R* se seleccionaron tres grupos de pacientes. El primer grupo estaba formado por 170 pacientes (104 mujeres y 66 hombres) con obesidad severa de inicio precoz cuya patogénesis podía ser atribuida a alteraciones genéticas. Todos presentaron un peso mayor a 3 desviaciones estándar a los 14 años de edad, la obesidad se mantuvo a lo largo de su vida de adultos y referían al menos dos familiares de primer o segundo grado con obesidad mórbida. El segundo grupo de estudio consistió en 77 mujeres no obesas, no relacionadas entre sí, que presentaban comportamiento por atracón y que habían sido diagnosticadas de BN o de Trastorno por Atracón. Finalmente, se incluyó un grupo de 20 mujeres delgadas diagnosticadas de AN.

Todos los pacientes eran españoles de raza Caucásica tratados en el Área de Salud de Badajoz que dieron su consentimiento informado para su participación en el estudio. El estudio recibió la aprobación del Comité de Bioética de la Universidad de Extremadura.

Los pacientes con TCA fueron diagnosticados por los clínicos de la Unidad de Trastornos Alimentarios (Centro de Salud de Valdepasillas, Badajoz) que participó en el estudio. El diagnóstico fue ciego al genotipo y se llevó a cabo usando la sección de trastornos alimentarios de la entrevista semiestructurada específica para DSM IV (First y cols., 1996).

Para minimizar en la medida de lo posible los factores de confusión en un grupo de enfermedades tan complejas como los TCA, se incluyeron en el estudio únicamente

aquellos casos reclutados en la Unidad de Trastornos Alimentarios de Badajoz con un diagnóstico de AN o BN, que recibían por primera vez un único tratamiento y cuyo seguimiento pudo llevarse a cabo por los profesionales de dicha Unidad.

4.1.2. Selección de controles

El grupo control estaba compuesto por 312 mujeres. Todos los sujetos eran de raza Caucásica tenían más de 18 años y pertenecían a la misma área geográfica que los pacientes. Las voluntarias fueron reclutadas entre los estudiantes y personal de la Facultad de Medicina y del Servicio Extremeño de Salud y fueron incluidas en el estudio tras obtener su consentimiento informado. Se llevaron a cabo entrevistas para garantizar que los sujetos control no recibían tratamiento psiquiátrico alguno, ni habían sido nunca diagnosticadas de ningún trastorno psiquiátrico. Se realizó además un examen físico para asegurar que el IMC estaba entre 20 y 25. Ninguna de las voluntarias mostró parámetros atropométricos indicativos de TCA.

4.2. EVALUACIÓN CLÍNICA DE LOS PACIENTES

Mediante examen retrospectivo de las historias clínicas, se recogieron valores de edad, año de inicio de la enfermedad, peso mínimo (menor peso desde que se inició la enfermedad), talla e IMC. Además, en la primera visita a la Unidad de Trastornos Alimentarios, se sometió a las pacientes a una entrevista para valorar la presencia de características psicopatológicas a través de dos cuestionarios: el *Eating Disorders Inventory Test-2* (EDI-2) y el *Symptom Checklist 90 Revisado* (SCL-90R). Aunque estos cuestionarios se diseñaron en países con culturas diferentes a la de nuestra sociedad, principalmente en países de habla inglesa, han sido validados previamente en la población española (Guimera y Torrubia, 1987; Derogaitis, 2002).

4.2.1. El test EDI-2

El *Eating Disorder Inventory* (EDI) (Garner y cols., 1983) o Inventario de Trastornos de la Conducta Alimentaria, es un inventario multifásico de 64 ítems. Se compone de ocho escalas que exploran las diferencias cognitivas y conductuales de los TCA. La última versión del EDI, el EDI-2 (Garner, 1991) que utilizamos en este trabajo, surgió de añadir 27 nuevos ítems a los 64 de la versión original. Esta modificación ha dado lugar al inventario actual compuesto por un total de 91 ítems agrupado en once escalas:

- Impulso u obsesión por adelgazar
- Sintomatología bulímica
- Insatisfacción corporal
- Sentimiento de insuficiencia
- Perfeccionismo
- Desconfianza
- Conciencia Interoceptiva
- Miedo a la madurez
- Ascetismo
- Regulación de los impulsos
- Inseguridad social

Cada ítem del EDI presenta 6 posibles respuestas que van de “siempre” a “nunca”, de las cuales sólo tres se puntúan de 1 a 3 (“a menudo”, “casi siempre” y “siempre”). Cuanto mayor es la puntuación obtenida, mayor es la frecuencia y severidad de los síntomas.

La fiabilidad *test-retest* de la escala validada en español puede considerarse aceptable y dichos resultados pueden ser indicativos de que probablemente la mayoría de las escalas del EDI están midiendo rasgos psicopatológicos estables (Corral y cols.,

1998). En el presente estudio utilizamos la versión del test EDI-2 adaptada a la población española de 1998 (Corral y cols., 1998).

4.2.2. El test SCL-90R

La Unidad de Investigación en Psicometría Clínica de la Universidad John Hopkins desarrolló el cuestionario SCL-90R a partir de su precursor *HSCL (Hopkins Symptom Checklist)*, una escala autoaplicada de valoración de síntomas que ha sido sometida a varias revisiones profundas (Derogaitis, 1977).

El inventario SCL-90R, que consta de 90 ítems que se responden en base a una escala de cinco puntos (0-4), evalúa una amplia gama de problemas psicológicos y síntomas de la psicopatología a través de tres índices globales (índice global de severidad, índice positivo de malestar, y total de síntomas positivos) y nueve dimensiones de síntomas primarios que comprenden un total de 83 artículos y que incluyen las dimensiones sintomáticas de:

- Somatización
- Obsesión-compulsión
- Sensibilidad interpersonal (sentimientos de inadecuación e inferioridad personal, autodesprecio)
- Depresión
- Ansiedad
- Hostilidad
- Ansiedad Fóbica
- Ideación Paranoide
- Psicoticismo

El SCL-90R incluye además 7 ítems que no se incorporan en las 9 dimensiones ya mencionadas pero que tienen relevancia clínica (poco apetito, problemas para

dormir, pensamientos acerca de la muerte, comer en exceso, despertar muy temprano, sueño intranquilo y sentimientos de culpa).

En este trabajo utilizamos la versión del test validada para la población española publicada en 2002 (Derogaitis, 2002).

4.3. SELECCIÓN DE SNPs

Los datos de SNPs presentes en la población europea (CEU) se obtuvieron de la página web del Proyecto Internacional de Mapa de Haplotipos (International Haplotype Mapping Project) (*http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap27_B36*). Analizamos la secuencia de codificación y las regiones 3' y 5' UTR adyacentes a los genes y los tag-SNPs (polimorfismos que pueden usarse como marcadores de una zona de variabilidad genética) fueron asignados mediante el software Haploview 4.2. Para la selección de los tag-SNPs se consideró un umbral de frecuencia del alelo variante del 10%, y un valor de r^2 de emparejamiento de al menos 0,80. De este modo, para el estudio del gen *NEGR1* (Ref. NC000001.10) se seleccionó un conjunto de 24 tag SNPs de los cuales tres (rs2630391, rs6696052, rs11209817) no pasaron el control de calidad con los métodos de genotipación. Todos los SNP estaban situados dentro de una región de 824,4 kb del gen *NEGR1* (posiciones 71910991 a 72735458 del cromosoma 1). Con el mismo procedimiento se seleccionaron 4 tag-SNPs del gen *BDNF* (Ref. NC_000011.10) que se encontraban situados contiguamente dentro del gen (cromosoma 11: 27676440–27743605). Todos los tag-SNPs seleccionados eran intrónicos (Tabla 7).

De manera adicional, se incluyeron en el estudio los polimorfismos rs6265 (Val66Met) y C-270T del gen *BDNF*, por ser dos SNPs muy estudiados en el campo de los TCA, habiéndose asociado en algunos trabajos con mayor riesgo de padecer alguna de estas patologías (Tabla 7).

SNP	POSICIÓN	GEN	ALELOS	MAF
rs1983121	71910991	*NEGR1*	A/T	0,209
rs7553624	71961690	*NEGR1*	G/T	0,209
rs357202	71978773	*NEGR1*	A/C	0,284
rs1041639	72016278	*NEGR1*	C/T	0,191
rs1413368	72058552	*NEGR1*	A/G	*0,005
rs928615	72077549	*NEGR1*	A/C	0,312
rs954299	72121585	*NEGR1*	G/T	0,456
rs2422021	72130161	*NEGR1*	C/T	0,441
rs10789322	72178928	*NEGR1*	A/G	0,298
rs12740031	72222487	*NEGR1*	A/G	0,301
rs6659202	72307420	*NEGR1*	A/T	0,244
rs591540	72331815	*NEGR1*	A/C	0,459
rs3851882	72345350	*NEGR1*	C/T	0,203
rs2114214	72388052	*NEGR1*	A/G	0,428
rs2186096	72440878	*NEGR1*	C/T	0,24
rs12409966	72539545	*NEGR1*	C/T	0,486
rs12091740	72553991	*NEGR1*	C/T	0,476
rs7517923	72614077	*NEGR1*	G/T	0,474
rs10493494	72670904	*NEGR1*	C/G	0,246
rs1026566	72709042	*NEGR1*	C/T	0,449
rs12137231	72735458	*NEGR1*	C/T	0,463
rs6265	27619916	*BDNF*	G/A (Val66Met)	0,21
rs11030102	27621596	*BDNF*	C/G	0,18
rs10835210	27635910	*BDNF*	A/C	0,44
rs16917237	27642383	*BDNF*	G/T	0,24
rs56164415 (C-270T)	27661737	*BDNF*	C/T	0,10
rs11030119	27668102	*BDNF*	A/G	0,24

Tabla 7: SNP genotipados del gen *NEGR1* y *BDNF*

MAF, *minor allele frequency* (frecuencia del alelo menor)

*Si bien el polimorfismo rs1413368 tiene en Caucásicos una frecuencia menor del umbral adoptado de 0.1 para la selección de tag SNPs, su frecuencia en otras poblaciones es mucho mayor (0.07-0.32). Dados los antecedentes de ciertas peculiaridades genéticas en la población Caucásica de la península Ibérica en comparación a grupos centro y norteeuropeos (Gervasini y cols., 2005) consideramos que debíamos incluirlo.

4.4. REACTIVOS

- Proteinasa K (Roche)
- SDS (Panreac)
- Fenol (Merck)
- Cloroformo (Merck)
- Isoamilalcohol (Merck)
- Isopropanol (Merck)
- Solución TE (Sigma Aldrich)
- Tris (Merck)
- Taq Polimerasa (Bioline, Ecogen)
- dNTP master mix (Ecogen)
- Oligonucleótidos (Thermo Fisher)
- Enzimas de restricción (Fermentas)
- Agarosa (Pronadisa)
- Bromuro de etidio 10 mg/ml (Sigma Aldrich)
- Cloruro de Magnesio (Sigma Aldrich)
- Ácido Bórico (Sigma Aldrich)
- 8-hidroxiquinoleína (Sigma Aldrich)
- Tampón de Hemólisis 10x (1 litro)
  - 82,9 g NH_4Cl (Merck)
  - 10 g KHCO3 (Merck)
  - 2 ml EDTA 0,5 M (SIGMA)

- Tampón S.E. (pH 8)
  - NaCl 75 mM (Merck)
  - EDTA 25 Mm (Sigma Aldrich)

4.5 MATERIAL

- Termociclador de 96 pocillos con gradiente G-storm (Gene technologies)
- Baño con termostato Precisterm (P-selecta)
- Centrífugas: Megafuge 1.0R (Heraeus instruments) y 5804 (Eppendorf)
- Tubos de centrífuga Falcon de 15 y 50 ml de capacidad
- Campana de flujo laminar
- Multiagitador magnético A-07 (SBS)
- Agitador y calentador Agimatic-E (P-selecta)
- Vórtex (VWR)
- pHmetro Crison Meterbasic 20+
- Balanza Precisa 1000C-3000D
- Estufa Heraeus b-5050 (Heraeus instruments)
- Trituradora de hielo Princess 2986TE
- Agitador Heidolph Reax-2
- Cámaras de electroforesis (Laboratorios Ecogen)
- Fuente de alimentación Apelex PS-304 para cámaras de electroforesis
- Aparato de fotodocumentación Transiluminator Quantum ST4
- Pipetas Eppendorf (1-10 µl, 10-100 µl, 20-200 µl, 100-1000 µl)
- Material de vidrio diverso (VWR)

4.6. MÉTODOS ANALÍTICOS

4.6.1. Protocolo para purificación de ADN genómico a partir de sangre entera

Para el estudio de genotipación, se extrajeron muestras de sangre (10 ml) de cada individuo en tubos de vidrio estériles con EDTA (Venoject, TerumoEurope N.V. 3001 Leuven, Bélgica) y se congelaron a -80ºC hasta su utilización. El ADN genómico se extrajo de los leucocitos (Neitzel, 1986) tal y como se describe a continuación:

1. Se descongela la sangre a temperatura ambiente durante 45 minutos.
2. Se depositan 10 ml de sangre en tubos de centrífuga de 50 ml sin faldón y se añaden 3 volúmenes de tampón de hemólisis. Se agitan los tubos a 5 rpm durante 20 minutos.
3. Se centrifuga la sangre a 3000 rpm durante 10 minutos a 4ºC con el fin de que los leucocitos sedimenten. En el sobrenadante quedan proteínas y restos de eritrocitos que se descartan mediante decantación.
4. Se añaden 15 ml más de tampón de hemólisis al sedimento leucocitario, Se vuelve a agitar durante otros 20 minutos y se centrifuga a 3000 rpm durante 10 minutos. Una vez finalizada la centrifugación, eliminamos el sobrenadante mediante decantación.
5. Añadimos 3 ml de solución SE al sedimento de leucocitos.
6. A esta suspensión se le añaden 200µl de Proteinasa K (10 mg/ml) y 200µl de SDS al 20 %.
7. Se agitan los tubos a temperatura ambiente durante 8-24 horas a 5 rpm.
8. Añadimos 5 ml de fenol (pH>7,8) y, suavemente, se agitan los tubos durante 20 minutos. Posteriormente, se centrifugan a 3000 rpm durante 10 minutos.
9. Después de la centrifugación observaremos dos fases claramente diferenciadas. Se extrae la fase acuosa (superior) y se deposita en otro tubo estéril.
10. En la fase acuosa, añadimos 5 ml de una mezcla de fenol/cloroformo (1:1). Agitamos durante 10 minutos y se centrifuga en las mismas condiciones del paso anterior. La fase acuosa que vuelve a quedar en la parte superior se pasa de nuevo a otro tubo estéril.
11. A esta fase acuosa añadimos ahora otros 5 ml de cloroformo/isoamilalcohol en proporción 24:1. Se vuelve a mezclar en el agitador y se centrifuga a 3000 rpm durante 10 minutos. Se extrae ahora la fase superior o acuosa y se pasa a un nuevo tubo estéril.

12. Añadimos 5ml de isopropanol a temperatura ambiente para que precipite el ADN.
13. Se mezcla bien ambas fases, sin movimientos bruscos, hasta la formación de un flóculo que será el ADN genómico. Dicho flóculo se extrae con una pipeta Pasteur y se lava con etanol 70% a -20ºC.
14. Los restos de etanol que quedan en el ADN se evaporan en una campana de vacío durante 30 minutos.
15. Se añaden 500 µl de solución T.E. y se almacena el ADN a 4ºC hasta su procesamiento.

La concentración de ADN y su pureza se determina midiendo la absorbancia en el espectrofotómetro según el siguiente procedimiento:

- Añadimos 700 µl de solución T.E. en una cubeta de cuarzo de 1 ml,
- Se mide la absorbancia a 260 nm y a 280 nm,
- Se añaden 5 µl de ADN y se vuelve a medir la absorbancia a las longitudes de onda ya señaladas,
- Se vuelven a añadir otros 5 µl de ADN a la cubeta y se repiten las mediciones,
- Se calcula la relación A260/A280, Un valor situado entre 1,82-2 indica que el ADN es puro. Si los valores quedan por debajo de este rango hay que volver a purificar de nuevo el ADN,

Como el factor de dilución empleado es de 705/5 (=141) y 710/10 (=71), se multiplica la densidad óptica a 260 por 141 o 71 y el resultado se multiplica por 50 (una A260 de 1000 equivale a 50 µg/ml de ADN genómico) para obtener la concentración de ADN en µg/ml,

4.6.2. Protocolo para precipitación de productos de PCR

En algunas digestiones enzimáticas es necesario precipitar el amplicón resultante de la PCR para eliminar restos de buffer de la reacción de amplificación que pueden interferir con la actividad endonucleasa de la enzima. Se realiza de la siguiente manera:

- Añadir a cada muestra de 20 µl de producto de PCR, 60,46 µl de mezcla de precipitado (15,5 µl $MgCl_2$ 2 Mm + 44,96 µl etanol 100%)
- Agitar brevemente en el vórtex
- Dejar 10 minutos en hielo
- Centrifugar 10 minutos a 13000 rpm
- Aspirar el sobrenadante
- Dejar secar en campana de vacío durante 15-30 minutos
- Añadir 5 µl de agua purificada
- Agitar en el vórtex durante 30 segundos

4.7. ANÁLISIS GENÉTICOS

A continuación, se describen las distintas técnicas de análisis genético utilizadas en este trabajo:

4.7.1. Análisis por PCR-RFLP (Polimorfismos de longitud de fragmentos de restricción)

Es una técnica que identifica cambios en la secuencia genética que ocurren en el sitio de corte de una enzima de restricción. Mediante este análisis obtenemos fragmentos de distinto tamaño en función de si el gen presenta en su secuencia los sitios de corte específicos de la enzima de restricción utilizada. Mediante esta técnica se analizaron los polimorfismos rs6265 y C-270T del gen *BDNF*.

Inicialmente, para analizar la presencia de las variantes genéticas de estudio en los sujetos participantes se realizaron amplificaciones por PCR (Reacción en Cadena de la Polimerasa). Las mezclas y condiciones de reacción de PCR, los oligonucleótidos utilizados, el tamaño de banda resultante y las enzimas de restricción utilizadas para analizar cada polimorfismo se detallan en la tabla 8.

	Rs6265 (Val66Met)	C-270T
Mezcla reacción PCR	• H_2O: 12,25 µl • Oligonucleótidos: 2 µl (cada uno) • dNTPs: 2,5 µl • Cl_2Mg (25 mM): 3,5 µl • Tampón amplificación 10x: 2,5 µl • Taq polimerasa: 0,25 µl • ADN: 1 µl	• H_2O: 9,75 µl • Oligonucleótidos: 2 µl (cada uno) • dNTPs: 2,5 µl • Cl_2Mg (25 mM): 2,5 µl • DMSO: 2,5 µl • Tampón ampllificación 10x: 2,5 µl • Taq polimerasa: 0,25 µl • ADN: 1 µl
Oligonucleótidos 5'→3'	AGG TGA GAA GAG TGA TGA CC CTG GAC GTG TAC AAG TCT GC	CAG AGG AGC CAG CCC GGT GC CTC CTG CAC CAA GCC CCA TTC
Condiciones PCR	Precalentamiento: 94ºC/4' 30 ciclos: • Desnaturalización:94ºC/30'' • Annealing: 60ºC/30'' • Extensión: 72ºC/30'' Retención:72ºC/7'	Precalentamiento: 95ºC/5' 30 ciclos: • Desnaturalización:95ºC/1' • Annealing: 59ºC/30'' • Extensión: 72ºC/30'' Retención:72ºC/7'
Tamaño amplicón	292 pb	223 pb
Enzima de restricción	NLa III	Hinf I

Tabla 8: Resumen de las mezclas y condiciones de reacción de PCR, oligonucleótidos utilizados, tamaño de banda resultante y las enzimas de restricción para el análisis de PCR-RFLP de los polimorfismos rs6265 y C-270T del gen *BDNF.*

Los productos de PCR obtenidos se sometieron a un proceso de purificación con etanol que eliminaba los oligonucleótidos no incorporados y los componentes del

tampón de amplificación. Tras esto, los amplicones se incubaron con sus correspondientes enzimas de restricción y los fragmentos resultantes se migraron mediante electroforesis en gel de agarosa entre el 1,2 y el 4%. En las tablas 9 y 10 se muestran los tamaños de los fragmentos (pb) obtenidos tras el proceso de restricción.

Rs6265 (Val66Met)		
Val/Val	Val/Met	Met/Met
157	157	
	80	80
62	62	62
58	58	58
15	15	15

C-270T		
C/C	C/T	T/T
127	127	127
78	78	80
	63	63
18	18	18
	15	15

Tablas 9 y 10: Fragmentos obtenidos (pb) tras el proceso de restricción.

4.7.2. Mass Array de Sequenom (iPLEX® Gold)

Los análisis genotípicos de los polimorfismos detallados en la tabla 11 se llevaron a cabo mediante la tecnología de PCR de extensión de una única base *Sequenom iPLEX-Gold* y la plataforma *MassARRAY MALDI-TOF*, basada en espectrometría de masas. Los análisis se realizaron en el Nodo de Santiago de Compostela del Centro Nacional de Genotipado (CEGEN-ISCIII).

El proceso se subdivide en tres pasos con dos reacciones de lavado intermedias (Figura 4). En primer lugar se amplifican los fragmentos de ADN que contienen las variantes de interés mediante una PCR multiplex y posteriormente, a partir de oligonucleótidos que hibridan en la región inmediatamente anterior del sitio polimórfico de interés, se realiza la extensión de una única base usando dideoxinucleótidos terminadores con masa modificada. Mediante la tecnología iPLEX® Gold todas las reacciones terminan tras una extensión de una única base que genera una diferencia de masa en el producto de extensión según la base añadida. Esta

diferencia de masa se detecta mediante espectrometría de masas MALDI-TOF (desorción/ionización mediante láser asistida por matriz acoplada a un analizador de tiempo de vuelo) (Gabriel y cols., 2009).

Polimorfismos	Gen
rs11030102, rs10835210, rs16917237, rs11030119	*BDNF*
rs1983121, rs7553624, rs357202, rs1041639, rs1413368, rs928615, rs954299, rs2422021, rs10789322, rs12740031, rs6659202, rs591540, rs3851882, rs2114214, rs2186096, rs12409966, rs12091740, rs7517923, rs10493494, rs1026566, rs12137231	*NEGR1*

Tabla 11: Polimorfismos de los genes *BDNF* y *NEGR1* analizados mediante la Mass Array de Sequenom, Tecnología iPLEX® Gold.

4.7.3. Secuenciación Sanger

Para el análisis de los polimorfismos del gen *MC4R* se analizó la secuencia bidireccional de ADN genómico que abarca toda la secuencia de codificación del único exón del gen *MC4R* (cromosoma 18: 58038564 - 58040001). La secuenciación se realizó en un analizador de ADN ABI 3130 (Applied Biosystems, Foster City, CA) usando el kit Big Dye Terminator Cycle Sequencing v3.1 (Applied Biosystems), según los protocolos recomendados por el fabricante. La secuencias obtenidas se analizaron con Sequencing Analysis v5.2 Software (Applied Biosystems) y Biosystems SeqScape v2.5 Software y se compararon con la secuencia de referencia (ENSG00000166603).

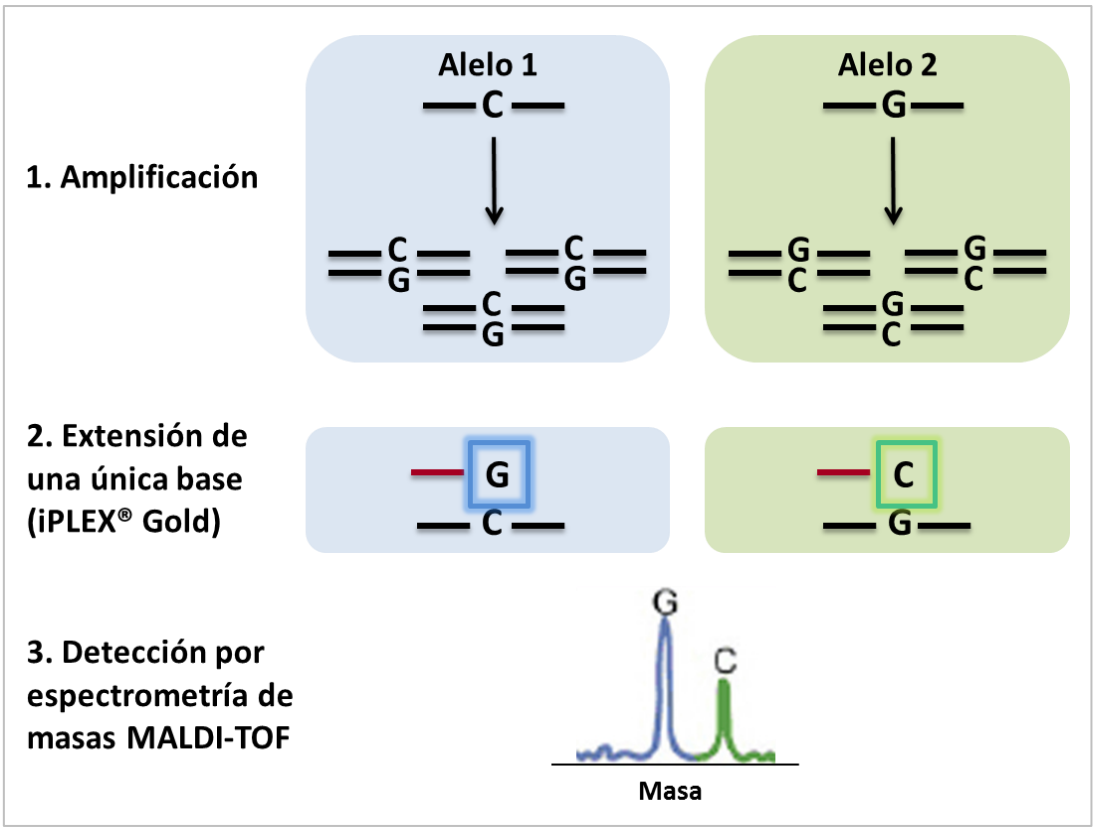

Figura 4: Representación esquemática de la Tecnología iPLEX® Gold y de la detección mediante espectrometría de masas MALDI-TOF.

Esta tecnología es una modificación del Método Sanger, y se basa en el empleo de dideoxinucleótidos que carecen del grupo hidroxilo del carbono 3', de manera que cuando uno de estos nucleótidos se incorpora a una cadena de ADN en crecimiento, esta cadena no puede continuar elongándose. La amplificación se realiza utilizando una pequeña proporción de dideoxinucleótidos marcados cada uno con un fluoróforo distinto, que al incorporarse de forma aleatoria durante el proceso de amplificación impiden la elongación posterior de la cadena y la dejan marcada su extremo 3´ para su detección posterior. Cada fragmento es separado en base a su tamaño mediante electroforesis capilar y la emisión de fluorescencia se detecta tras la excitación mediante un láser.

4.8. MÉTODOS ESTADÍSTICOS

La potencia estadística del tamaño de la muestra fue evaluada con un modelo genético asumiendo un modelo de herencia log aditivo y analizando la frecuencia de portadores de los alelos variantes con un tamaño del efecto arbitrariamente establecido en 2,0 (Error tipo I=0,05). Con el tamaño de muestra disponible y las frecuencias conocidas de los alelos menores, el poder estadístico para detectar asociaciones con variables categóricas para los distintos genes analizados estuvo entre 0,80 y 0,99 (Quanto software v. 1.2.4. University of Southern California).

Las comparaciones de las frecuencias alélicas y genotípicas de los genes en los casos y controles se realizaron con la prueba exacta de Fisher o el test χ^2, según hubiera dos o más grupos de comparación respectivamente.

La asociación entre los polimorfismos del gen *BDNF* estudiados y el riesgo de desarrollar AN o BN se estimó mediante Odds Ratio (OR) con un intervalo de confianza del 95% (IC). Las distribuciones de las variables cuantitativas entre los diferentes genotipos se compararon mediante t-test o ANOVA, según procediese, y los resultados fueron corregidos por el método de Bonferroni de comparación múltiple ajustando por el número de SNPs estudiados. Con el fin de evaluar la asociación entre las variantes y los parámetros fisiológicos y psicopatológicos, y después del estudio de los resultados por varios modelos de herencia usando la plataforma SNPstats (Sole y cols., 2006) (http://bioinfo.iconcologia.net/snpstats/start.htm), se escogió el modelo recesivo para los análisis. Estos análisis se realizaron mediante regresión logística ajustada por edad utilizando el paquete estadístico para las Ciencias Sociales (SPSS) versión 15.0 para Windows (SPSS Inc,, Chicago, Illinois, EEUU) o bien con el paquete *SNPassoc* para el software *R* (Gonzalez y cols., 2007). Los resultados obtenidos también fueron corregidos mediante el Test de Bonferroni de comparaciones múltiples.

La plataforma SNPstats se utilizó asimismo para estimar las frecuencias generales de los haplotipos del gen *BDNF* y para obtener los datos de desequilibrio de ligamiento. Los bloques haplotípicos del gen *NEGR1* fueron definidos mediante el programa Haploview v 4.2. Para refinar la construcción de haplotipos e identificar las regiones centrales con potencial de afectar tanto a la susceptibilidad de padecer TCA como a los parámetros fisiológicos y psicopatológicos que presentan los pacientes, se utilizó un enfoque de *sliding window* (ventana deslizante) para construir haplotipos sucesivos y adyacentes cada 3 SNPs. Posteriormente, las asociaciones caso/control y las asociaciones cuantitativas se evaluaron mediante el software PLINK v. 1.07 usando como punto de corte 0,1 en la frecuencia de haplotipos (Purcell y cols., 2007).

El diseño del estudio del gen *MC4R* fue distinto a los de los genes anteriores. En este caso se llevó a cabo un T-test para analizar diferencias en los parámetros antropométricos y psicopatológicos entre portadores y no portadores de mutaciones en el gen (SPSS Inc., Chicago, Illinois, EEUU). Para evaluar el impacto de las mutaciones del gen *MC4R* en la función de la proteína se utilizaron dos software de predicción: *PolyPhen-2 (*Polymorphism Phenotyping v2*)* (Adzhubei y cols., 2013) y *SIFT (*Sorting Tolerant From Intolerant*)* (JCV Institute, La Jolla, California, USA) (Kumar y cols., 2009).

En todos los casos, se consideraron diferencias estadísticamente significativas cuando los valores de p eran menores de 0,05 ($p<0,0023$ para el gen *NEGR1* y $p<0,0083$ para el gen *BDNF* tras corrección por Bonferroni).

5. RESULTADOS

A continuación se exponen los resultados más relevantes que se obtuvieron en el presente trabajo y que vienen reflejados en las tres publicaciones anexas a esta tesis. Los resultados se agrupan en tres epígrafes, cada uno correspondiente a los tres estudios consecutivos que se realizaron.

5.1. ESTUDIO DEL GEN *BDNF*

5.1.1. Estudio descriptivo de la población de estudio

En la tabla 12 se muestran los datos descriptivos y las variables clínicas de los pacientes (AN y BN) y controles incluidos en el estudio del gen *BDNF*. El peso mínimo medio (44,28 ± 6,08 kg) y el IMC (17,19 ± 2,01) de las pacientes con AN fueron significativamente menores que los que presentaban los controles ($p < 0,05$). Además los pacientes con BN presentaron puntuaciones más altas que los pacientes con AN en las distintas escalas utilizadas para la evaluación psicométrica.

	AN	BN	Controles	p
Edad, años	18.9 ± 6.20	20.9 ±8.11	21,90 ± 7,01	NS
Altura, m	1,60 ± 0,07	1,61 ± 0,06	1,62 ± 0,07	NS
Peso, kg	44,28 ± 6,08	59,16 ± 13,65	58,3 ± 10,12	<0,05[a]
IMC, kg/m^2	17,19 ± 2,01	22,71 ± 5,03	21,99 ± 4,25	<0,05[a]
Edad de inicio, años	17,0 ± 5,0	18,0 ± 5,0		
Puntuación total EDI-2	88,84 ± 45,53	117,27 ± 39,14		<0,0001[b]
Puntuación GSI (SCL-90R)	1,62 ± 0,85	1,96 ± 0,82		<0,05[b]
Puntuación PST (SCL-90R)	63,33 ± 21,91	71,11 ± 17,07		<0,05[b]
Puntuación PSDI (SCL-90R)	2,18 ± 0,64	2,4 ± 0,62		<0,05[b]

Tabla 12: Datos descriptivos y variables clínicas de los pacientes y controles.
[a]valor de p significativo para la comparación entre controles y pacientes del grupo AN.
[b]valor de p significativo para la comparación entre pacientes de AN y BN.

5.1.2. Análisis de SNPs individuales

Con el fin de simplificar la exposición de los resultados y mostrar los datos más relevantes del estudio, sólo se hará referencia a las asociaciones que fueron significativas después de corregir mediante el test de Bonferroni de comparaciones múltiples. El resto de resultados se muestra en el primero de los tres artículos adjuntos.

Los análisis de genotipación no pudieron completarse en diez muestras para el SNP rs11030119, cuatro para el rs16917237 y una para el rs11030102 en el grupo control. Los datos que se obtuvieron para el resto de muestras de pacientes y controles fueron válidos.

Las frecuencias de los seis polimorfismos estudiados en el grupo control se encontraban en equilibrio de Hardy-Weinberg ($p>0.05$).

Ninguno de los SNPs estudiados se asoció con mayor riesgo de padecer AN o BN (Tabla 13). En relación a los parámetros fisiológicos, los análisis llevados a cabo siguiendo un modelo de herencia recesivo, revelaron que el genotipo homocigoto mutante TT del polimorfismo rs16917237 estaba asociado con mayor IMC ($p<0,01$) y con mayor peso mínimo ($p<0,01$) en pacientes con BN (Tabla 14).

	AN (n =106)		BN (n = 63)	
	GG+GT	TT	GG+GT	TT
Peso, kg	44,25 ± 6,15	46 ± 6,41	57,93 ± 13,02	74,63 ± 16,58*
Altura, m	1,61 ± 0,07	1,59 ± 0,03	1,61 ± 0,06	1,61 ± 0,03
IMC, kg/m^2	17,12 ± 1,92	18,34 ± 3,13	22,23 ± 4,77	28,94 ± 6,22*

Tabla 14. Asociación entre el polimorfismo rs16917237 del gen BDNF con parámetros antropométricos.

Polimorfismos	Controles (%)	AN (%)	OR (CI)	p valor	BN (%)	OR (CI)	p valor
rs11030102							
C/C	206 (66,2)	69 (65,1)	Referencia		37 (58,7)	Referencia	
C/G	96 (30,9)	31 (29,2)	0,96 (0,59-1,57)	0,90	24 (38,1)	1,39 (0,79-2,46)	0,3
G/G	9 (2,9)	6 (5,7)	1,99 (0,68-5,79)	0,23	2 (3,2)	1,24 (0,56-5,96)	1
rs16917237							
G/G	182 (59,1)	60 (56,6)	Referencia		32 (50,8)	Referencia	
G/T	105 (34,1)	39 (36,8)	1,13 (0,70-1,80)	0,63	27 (42,9)	1,46 (0,83-2,57)	0,24
T/T	21 (6,8)	7 (6,6)	1,01 (0,41-2,50)	1	4 (6,3)	1,08 (0,35-3,36)	1
rs11030119							
G/G	168 (55,6)	61 (57,5)	Referencia		30 (47,6)	Referencia	
G/A	122(40,4)	36 (34)	0,81 (0,51-1,30)	0,41	29 (46)	1,33 (0,76-2,33)	0,39
A/A	12 (4)	9 (8,5)	2,06 (0,83-5,14)	0,13	4 (6,3)	1,87 (0,56-6,17)	0,48
rs10835210							
C/C	106 (34)	33 (31,1)	Referencia		26 (41,3)	Referencia	
A/C	137 (43,9)	55 (51,9)	1,29 (0,78-2,13)	0,38	28 (44,4)	0,83 (0,46-1,50)	0,65
A/A	69 (22,1)	18 (17)	0,60 (0,26-1,37)	0,63	9 (14,3)	0,53 (0,23-1,20)	0,18
rs6265							
Val/Val	195 (62,5)	62 (58,8)	Referencia		34 (54)	Referencia	
Val/Met	103 (33)	38 (35,9)	1,16 (0,73-1,85)	0,55	26 (41,3)	1,45 (0,82-2,54)	0,24
Met/Met	14 (4,5)	6 (5,7)	1,35 (0,50-3,66)	0,59	3 (4,8)	1,23 (0,33-4,50)	1
rs56164415 (-207C/T)							
C/C	281 (90,1)	96 (90,6)	Referencia		52 (82,5)	Referencia	
C/T	31 (9,9)	10 (9,4)	0,94 (0,45-2)	1	11 (17,5)	1,92 (0,91-4,05)	0,12

Tabla 13. Frecuencias genotípicas de los polimorfismos analizados en pacientes con AN y BN y en sujetos control. Valores de Odds ratio (OR) con intervalos de confianza (IC) del 95 %.

Respecto a los valores arrojados por los test psicológicos, el estudio de las variantes genéticas del *BDNF* mostró que las pacientes del grupo AN que eran portadoras del genotipo -270CC, presentaban puntaciones significativamente más altas que las portadoras del genotipo CT en la escala de *Desconfianza Interpersonal* del test EDI-2 (6,91 ± 4,89 vs 2,67 ± 3,12, Tabla 15). No hubo pacientes con genotipo homozigoto mutante TT.

	rs56164415 (C-270T)	
	C/C	**C/T**
EDI-2		
DT	11,19 ± 6,45	12,33 ± 5,94
B	3,02 ± 4,59	1,78 ± 3,23
BD	13,04 ± 8,5	11,67 ± 5,98
I	10,98 ± 8,02	6,56 ± 8,08
P	5,91 ± 3,95	6,11 ± 5,82
ID	***6,91 ± 4,89**	2,67 ± 3,12
IA	9,63 ± 7,2	6,11 ± 6,72
MF	8,46 ± 5,4	6,33 ± 4,8
A	6,56 ± 4,48	5,78 ± 4,12
IR	7,24 ± 5,88	5,78 ± 6,98
SI	7,6 ± 5,47	4,33 ± 4,06
Total	90,55 ± 45,22	69,44 ± 45,45
SCL-90R		
GSI	1,6 ± 0,84	1,4 ± 0,88
PST	62,55 ± 21,25	62,11 ± 27,9
PSDI	2,2 ± 0,64	1,85 ± 0,61

Tabla 15. Asociación entre SNP rs56164415 (C-270T) y las diferentes escalas de los cuestionarios EDI- 2 y SCL-90R en pacientes con AN. DT (Drive for Thinness, Obsesión por la Delgadez); B (Bulimia); BD (Body Dissatisfaction Insatisfacción Corporal); I (Ineffectiveness, Ineficacia); P (Perfectionism, Perfeccionismo); ID (Interpersonal Distrust, Desconfianza Interpersonal; IA (Interoceptive Awareness, Conciencia Interoceptiva); MF (Maturity Fears, Miedo a la Madurez), A (Asceticism, Ascetismo); IR (Impulse Regulation, Impulsividad); SI (Social Insecurity, Inseguridad Social); GSI (Global Severity Index, Índice de Severidad Global); PST (Positive Symptom Total, Total Síntomas Positivos); PSDI (Positive Symptom Distress Index, Índice Positivo de Malestar).

De la misma manera, las pacientes del grupo de BN que eran portadores de este mismo genotipo (-270CC), presentaban valores más altos en dos de las tres escalas globales del cuestionario SCL-90R: GSI (2,08 ± 0,81 vs 1,44 ± 0,68, Tabla 16) y PST (73,31 ± 15,69 vs 59,91 ± 20,0; Tabla 16). Finalmente, las pacientes con BN portadoras del genotipo mutante homocigoto del polimorfismo rs11030102 presentaban puntuaciones significativamente más altas en la escala de Inseguridad Social que las pacientes que presentaban al menos un alelo de tipo salvaje (7,83 ± 4,75 vs 15,0 ± 1,41; Tabla 16).

	rs56164415 (C-270T)		rs11030102	
	C/C	*C/T*	*C/C + G/C*	*G/G*
EDI-2				
DT	14,35 ± 4,82	16 ± 4,36	14,6 ± 4,81	16 ± 2,83
B	9,88 ± 7,23	11,09 ± 7,8	10,2 ± 7,3	7 ± 8,49
BD	19,14 ± 8,01	20,64 ± 7,45	19,45 ± 7,99	18 ± 4,24
I	12,27 ± 8,18	12,27 ± 7,55	11,98 ± 7,85	21 ± 11,31
P	6,55 ± 4,57	6,91 ± 4,06	6,63 ± 4,47	6 ± 5,66
ID	6,41 ± 4,64	6,55 ± 3,33	6,3 ± 4,26	10,5 ± 9,19
IA	14,1 ± 6,87	15,82 ± 5,49	14,22 ± 6,66	20 ± 1,41
MF	9,49 ± 6,03	7,27 ± 4,82	9,03 ± 5,91	11 ± 5,66
A	7,76 ± 4,41	6,91 ± 2,84	7,5 ± 4,19	11 ± 0,0
IR	9,98 ± 6,55	8,55 ± 6,06	9,7 ± 6,53	10,5 ± 3,54
SI	7,76 ± 4,82	9,45 ± 4,95	7,83 ± 4,75	***15,0 ± 1,41**
Total	117,71 ± 40,25	121,45 ± 35,92	117,45 ± 39,32	146 ± 35,36
SCL-90R				
GSI	***2,08 ± 0,81**	1,44 ± 0,68	1,95 ± 0,83	2,49 ± 0,33
PST	***73,31 ± 15,69**	59,91 ± 20	70,75 ± 17,28	77,5 ± 13,44
PSDI	2,48 ± 0,64	2,09 ± 0,47	2,39 ±0,63	2,91 ± 0,12

Tabla 16. Asociación entre el SNP rs56164415 (C-270T) y las diferentes escalas de los cuestionarios EDI- 2 y SCL-90R en pacientes con BN.

5.1.3. Análisis de haplotipos

Para varios de los SNPs analizados en el gen BDNF se observó un alto grado de ligamiento en la población de estudio. Los datos de este análisis de ligamiento (D, D', r y p) están representados en la Figura 5. En el análisis del haplotipos realizado mediante el enfoque de ventana deslizante usando 3 SNPs consecutivos (3-SNP *sliding-window approach*), destaca la presencia de 4 locus de interés:

- Locus 1, incluye los polimorfismos rs6265, rs11030102 y rs10835210
- Locus 2, incluye los polimorfismos rs11030102, rs10835210 y rs16917237
- Locus 3, incluye los polimorfismos rs10835210, rs16917237 y C-270T
- Locus 4, incluye los polimofismos rs11030119, rs16917237 y C-270T.

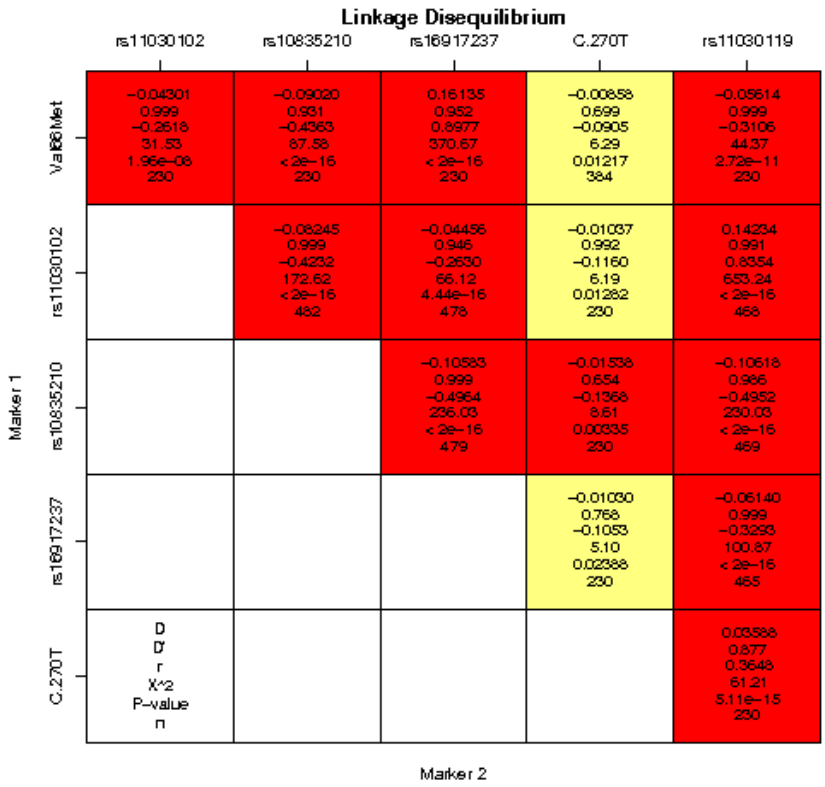

Figura 5: Datos del desequilibrio de ligamiento de los SNPs del gen *BDNF*.

Al igual que en el estudio de SNPs individuales comentado previamente, no se encontraron diferencias relevantes en la distribución de estos haplotipos entre los

grupos de pacientes y los sujetos control. Sólo el haplotipo GCG en el locus 4 fue ligeramente más frecuente en el grupo de control (frecuencia = 0,527) que en el de pacientes con BN (frecuencia = 0,428; p=0,044).

A continuación, se realizó el análisis de asociación de los haplotipos identificados en relación a los parámetros fisiológicos y psicopatológicos que presentaban las pacientes. En relación a los parámetros fisiológicos, no se identificó ninguna asociación significativa, sin embargo, el análisis de los rasgos psicopatológicos sí reveló algunas asociaciones de interés (Tablas 17 y 18). En cualquier caso, solo la asociación del haplotipo CGC del locus 3 con la escala *GSI* en pacientes con AN mantuvo la significación estadística después de ajustar mediante la corrección de Bonferroni para los cuatro haplotipos (p<0,0125).

SCL90-R	Haplotipo	BETA	r^2	p
GSI	CGT	-0,0737	4,567e-04	0,8329
	CTC	-0,2299	0,02774	0,0977
	AGC	-0,108	0,007311	0,3976
	CGC	**0,3401**	**0,07197**	**0,006961**
PST	CGT	3,749	0,00177	0,6777
	CTC	-6,05	0,02879	0,09145
	AGC	-1,599	0,002398	0,6285
	CGC	**6,798**	**0,04305**	**0,03833**
PSDI	CGT	-0,2902	0,01243	0,272
	CTC	-0,07516	0,005168	0,4795
	AGC	-0,06929	0,005278	0,4749
	CGC	**0,1996**	**0,04318**	**0,03903**

Tabla 17: Correlación entre los haplotipos del gen *BDNF* de la combinación de polimorfismos rs10835210 / rs16917237 / C-270T y los rasgos psicopatológicos medidos mediante el test SCL-90R en AN. El haplotipo estadísticamente significativo se muestra en negrita.

SCL90-R	Haplotipo	BETA	r^2	p
GSI	**CGT**	**-0.6487**	**0.09039**	**0.01758**
	CTC	-0.02548	3.581e-04	0.8839
	AGC	0.01084	8.028e-05	0.9449
	CGC	0.2425	0.03541	0.143
PST	**CGT**	**-13.52**	**0.09108**	**0.0171**
	CTC	-0.4788	2.933e-04	0.8949
	AGC	2.001	0.006343	0.5383
	CGC	2.994	0.01251	0.3868
PSDI	**CGT**	**-0.3923**	**0.05824**	**0.06099**
	CTC	-0.02895	7.435e-04	0.8348
	AGC	0.02479	7.285e-04	0.8364
	CGC	0.1377	0.01993	0.2778

Tabla 18: Correlación entre los haplotipos del gen *BDNF* de la combinación de polimorfismos rs10835210 / rs16917237 / C-270T y los rasgos psicopatológicos medidos mediante el test SCL-90R en BN. El haplotipo estadísticamente significativo se muestra en negrita.

A modo de resumen, se exponen los principales resultados del gen *BDNF*:

- Ninguno de los SNPs analizados de manera individual y/o conjunta, se asoció con mayor riesgo de padecer AN o BN en la población de estudio.
- En relación al análisis de cada SNPs por individual, se encontró una asociación del genotipo rs16917237TT con mayor IMC y peso en pacientes con BN. Las escalas globales *GSI* y *PTS* del test SCL-90R y la escala de *Inseguridad Social* del EDI-2 se vieron afectadas por los genotipos -270CC y rs11030102 GG, respectivamente en el grupo de BN. En el grupo de AN tan solo el genotipo homocigoto mutante -270CC se asoció con puntuaciones más elevadas en la escala de *Desconfianza Interpersonal*.
- El análisis de haplotipos mediante ventana deslizante reveló la existencia de un haplotipo asociado con la escala *GSI* en pacientes con AN.

5.2. ESTUDIO DEL *NEGR1*

5.2.1. Estudio descriptivo de la población de estudio

En la tabla 12 anterior se muestran las características clínicas y descriptivas de los sujetos incluidos en este trabajo, que fueron los mismos que para el estudio del gen *BDNF*.

5.2.2. Análisis de SNPs individuales

Siguiendo el mismo esquema del apartado anterior, sólo se hará referencia a las asociaciones que fueron significativas después de corregir mediante el test de Bonferroni de comparaciones múltiples. El resto de resultados se puede consultar en el segundo artículo adjunto al final de la tesis.

Ninguno de los SNPs analizados de manera individual se asoció con mayor riesgo de padecer TCA o con alguno de los parámetros antropométricos. Sin embargo, se observó una clara asociación, en el grupo de BN, de cuatro variantes genéticas del gen *NEGR1* con varias escalas del cuestionario EDI-2. Dos de estos SNPs, se encontraban en completo desequilibrio de ligamiento (rs12740031 y rs10789322) y se asociaron con la escala de *Conciencia Interoceptiva* y con la puntuación total del cuestionario EDI-2, con diferencias significativas entre los tres genotipos (homocigoto normal, homocigoto mutante y heterocigoto) (Tabla 19). De la misma manera, los SNPs rs6659202 y rs591540 se asociaron con las escalas *Inefectividad* y *Obsesión por Adelgazar* respectivamente (Tabla 19).

	rs10789322/rs12740031†			rs6659202			rs591540		
Escala	A/A	A/G	G/G	A/A	A/T	T/T	A/A	A/C	C/C
DT	15,25 ± 2,50	12,95 ± 1,04	15,61 ± 0,73	10,0 ± 0,0	13,83 ± 0,90	15,53 ± 0,80	***16,83 ± 0,55**	15,42 ± 0,72	10,72 ± 14,76
I	5,75 ± 2,1	9,27 ± 1,77	14,83 ± 1,20	***15,78 ± 1,4**	8,69 ± 1,20	4,0 ± 0,0	12,5 ± 1,37	12,97 ± 1,43	10,29 ± 2,37
IA	***17,25 ± 0,92**	11,32 ± 13,40	5,75± 0,95	3,0 ± 0,0	12,69 ± 1,16	16,31 ± 1,13	16 ± 1,12	14,75 ± 1,13	12,14 ± 2,06
EDI-2	***133,89 ± 5,77**	99,41 ± 7,64	83,00 ± 17,30	36,0 ± 0,0	108,17 ± 7,24	130,19 ± 6,13	128,50 ± 8,95	121,42 ± 6,43	101,86 ± 11,97

Tabla 19. Asociación entre SNPs del gen *NEGR1* (modelo codominante) y diferentes escalas del cuestionario EDI- 2 en pacientes con BN.

†rs10789322 (A/G) and rs12740031 (A/G) se encontraban en complete desequilibrio de ligamiento en pacientes con BN, por lo que se muestran los mismos valores para ambos.

*p <0.05 para la diferencia entre los tres genotipos después de corregir mediante test de Bonferroni de comparaciones multiples

5.2.3. Análisis de haplotipos

Al igual que en el estudio individual de cada marcador, no se obtuvieron diferencias relevantes en la distribución de los diferentes haplotipos entre los pacientes y los sujetos control.

En cambio, de nuevo se detectaron diferencias significativas en relación a la evaluación psicométrica. En concreto, se detectó una clara influencia de varios haplotipos del gen *NEGR1* en el grupo de BN. El estudio de las combinaciones de los polimorfismos reveló la existencia de dos bloques haplotípicos. El primer bloque, de 17 kb, incluía dos SNPs (rs7553624 y rs357202) y el segundo de 100 kb incluía cuatro SNPs (rs954299, rs2422021, rs10789322, rs12740031) (Figura 6). El haplotipo GCAA del segundo bloque mostró una fuerte asociación con puntaciones totales más elevadas en el cuestionario EDI-2.

Para investigar en mayor profundidad esta observación, se realizó un análisis de ventana deslizante de tres SNPs consecutivos, y evaluar así la asociación de los 19 loci resultantes con las diferentes escalas. De este análisis se obtuvo una región central en el gen, que abarcaba una región de 210 kb, que se asociaba con puntuaciones más elevadas en las escalas *Obsesión por Delgadez, Bulimia, Conciencia Interoceptiva* e *Inefectividad*, y también con una mayor puntuación total en el cuestionario EDI-2 (Figura 7). Es importante señalar que esta región incluía los 4 SNP que formaban el bloque 2 descrito anteriormente. Tan solo otra combinación (rs12091740, rs7517923, rs10493494), situada en una región anterior, se asoció con aumentos en la escala *Conciencia Interoceptiva* y en la puntuación total del cuestionario EDI-2 (Figura 7).

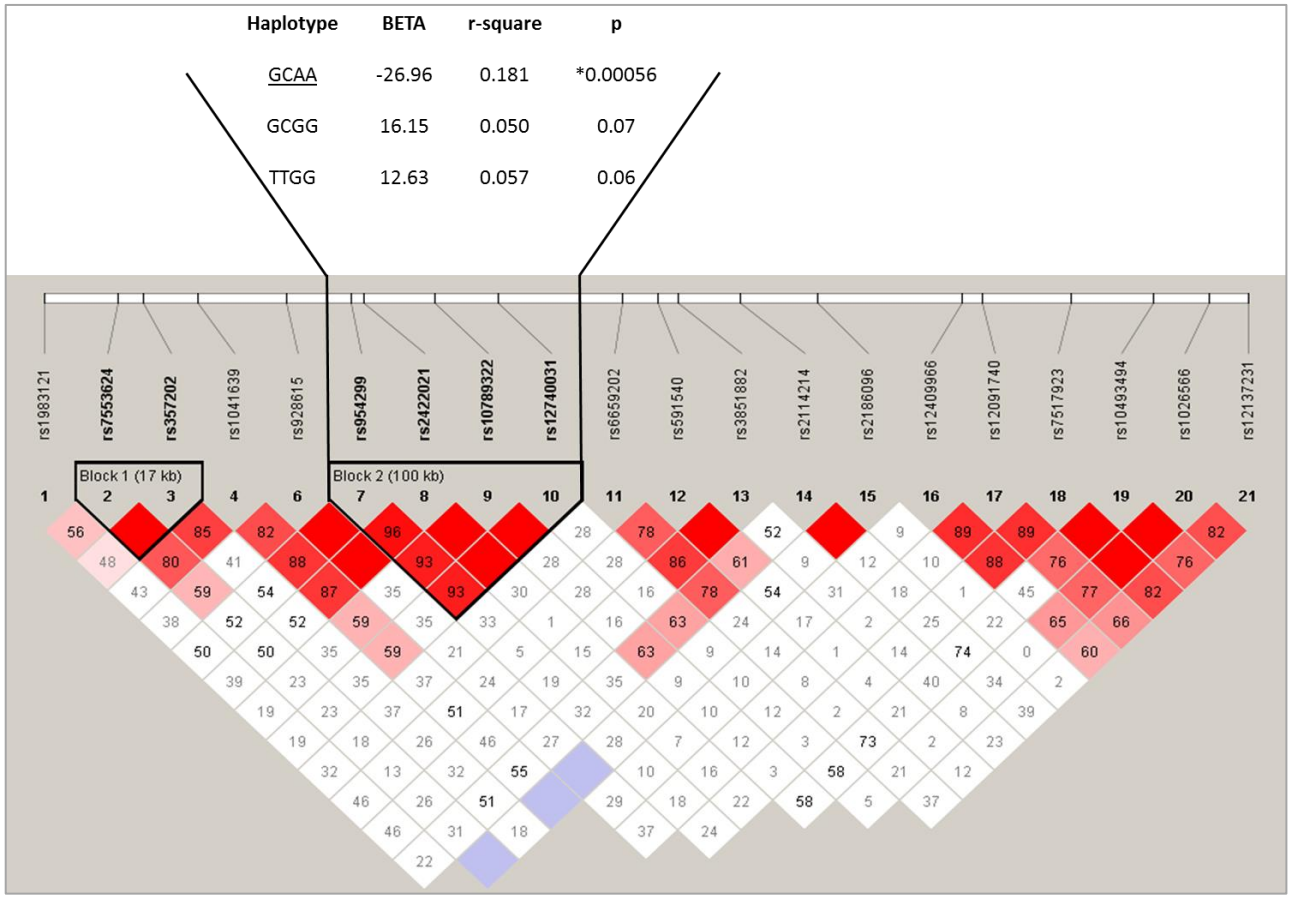

Figura 6: Bloques 1 y 2 del análisis de haplotipos.

*El haplotipo GCAA asociado con mayor puntuación total del cuestionario EDI-2.

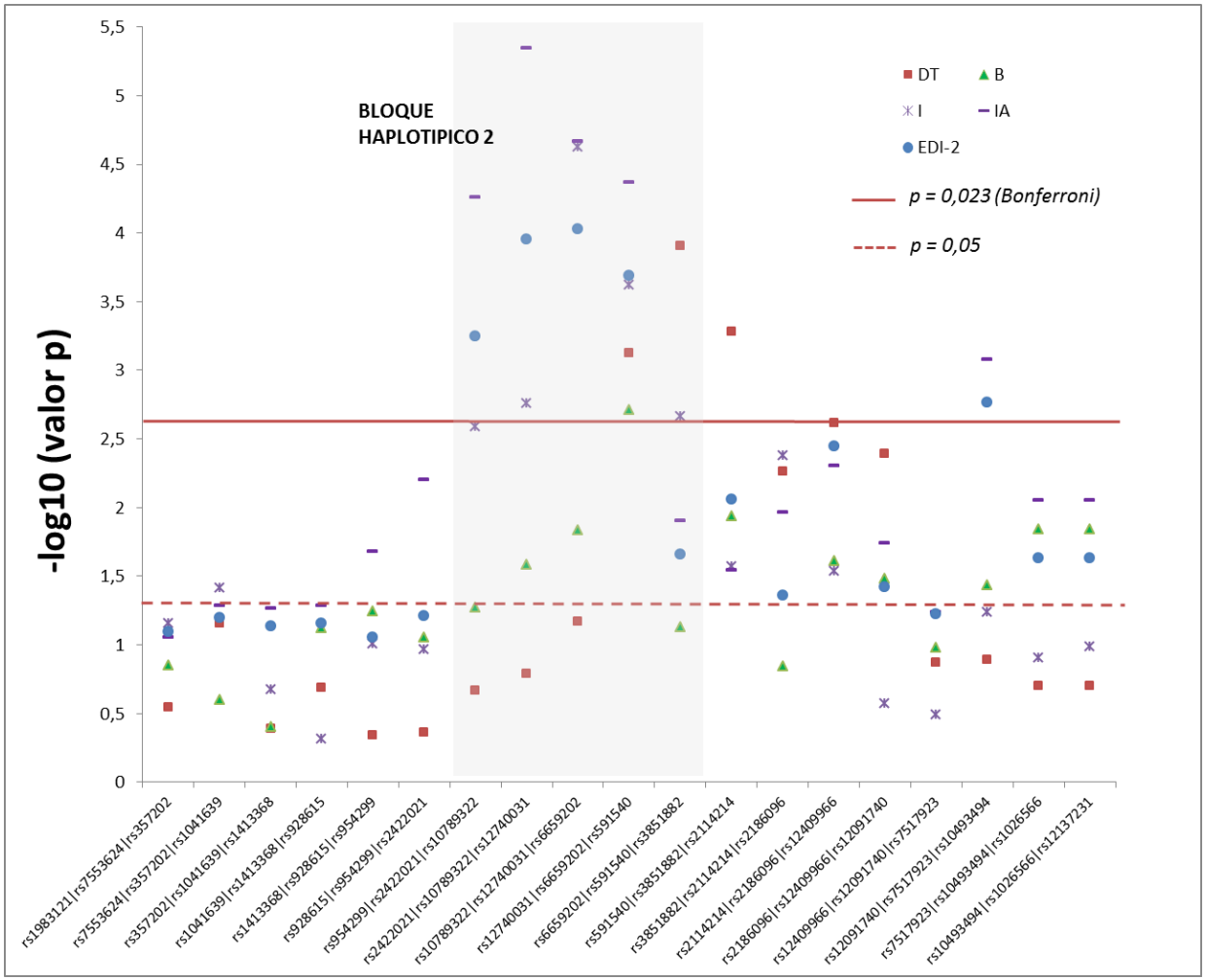

Figura 7: Análisis de haplotipos mediante *ventana deslizante.* Se resalta la región central del gen *NEGR1*, asociada con varias escalas del cuestionario EDI2

Los principales hallazgos derivados del análisis de los polimorfismos del gen *NEGR1* fueron por lo tanto que:

- No se detectó ninguna asociación significativa entre los polimorfismos analizados, tanto de manera individual como conjunta, con un mayor riesgo de padecer TCA o de presentar parámetros antropométricos alterados.
- Varias variantes génicas mostraron una clara influencia en los parámetros psicológicos de las pacientes con BN. Lo más destacable de los resultados obtenidos a este respecto fue la identificación de una región central en el gen que se asocia con mayores puntuaciones en las escalas *Obsesión por Delgadez, Bulimia, Conciencia Interoceptiva e Inefectividad*, y con una mayor puntuación total en el cuestionario EDI-2.
- En el grupo de AN no se identificó ninguna asociación genética significativa.

5.3. ESTUDIO DEL GEN *MC4R*

Los parámetros antropométricos y psicopatológicos de los participantes incluidos en el estudio del gen *MC4R* se resumen en la tabla 20. Se observaron diferencias significativas en el peso, IMC y valores totales de los cuestionarios EDI-2 y SCL-90R entre los dos grupos de paciente con TCA.

El diseño de este estudio fue diferente al de los dos anteriores. Se seleccionaron tres grupos de población: (i) un grupo de pacientes con obesidad mórbida de inicio precoz formado por 104 mujeres y 66 hombres; (ii) 77 pacientes no obesas con comportamiento por atracón diagnosticadas de BN o de trastornos por atracón y, a efectos comparativos, (iii) 20 mujeres con bajo IMC diagnosticadas de AN.

En los pacientes obesos, se identificaron diez variantes que producían cambio de aminoácido en doce sujetos (Tabla 21) y la frecuencia total de las variantes fue del 3,8%. Todos los portadores eran heterocigotos para un cambio de nucleótido único, excepto un paciente heterocigoto doble portador de los polimorfismos S127L y G252S.

	Obesos (n=170)	Comportamiento atracón (n=77)	AN (n=20)	p
Edad inicio, años	16,01 ± 13,20	17,71 ± 4,81	15,50 ± 2,30	
Mujeres (%)	61,2	100	100	
Altura, m	1,57 ± 0,14	1,61 ± 0,05	1,60 ±0,07	
Peso, kg	93,81 ± 28,37	61,33 ± 17,91	42,16 ± 6,18	<0,0001[a]
IMC	37,13 ± 6,64	23,40 ± 6,20	16,40 ± 1,59	<0,0001[a]
EDI-2, puntuación total	-	117,5 ± 38,53	86,72 ± 36,40	<0,05[a]
SCL-90R GSI	-	1,96 ± 0,81	1,37 ± 0,73	<0,05[a]

Tabla 20. Datos descriptivos y variables clínicas de los sujetos incluidos en el estudio.
[a]valor de p significativo para la comparación entre pacientes del grupo comportamiento por atracón y pacientes del grupo AN.

En el grupo de pacientes con comportamiento de atracones, se identificaron sólo dos pacientes (diagnosticadas de BN) que portaban mutaciones en *MC4R* (polimorfismo I251L; Tabla 21). La frecuencia total de polimorfismos en este grupo fue significativamente menor que la obtenida en el grupo de obesos (1,3% vs 3,8%; p<0,05) (Tabla 21). En el grupo de pacientes con AN no se detectó ninguna mutación.

			Obesos	Comportamiento de atracón
Cambio de nucleótido	**Referencia HGMD**	**Cambio de AA**	**N (%)**	**N (%)**
c.20G>A	CM035774	R7H	1 (0,29)	-
c.95G>A	CM070989	G32E	1 (0,29)	-
c.227A>G	CM085524	H76R	1 (0,29)	-
c.307G>A	CM030481	V103I	4 (1,18)	-
c.380C>T	CM030234	S127L	1(0,29)	-
c.439A>G	-	R147G	1 (0,29)	-
c.449C>T	CM003759	T150I	1(0,29)	-
c.751A>C	CM030483	I251L	1(0,29)	2 (1,3)
c.754G>A	CM990835	G252S	1(0,29)	-
c.968G>A	-	G323E	1(0,29)	-
		Total	13 (3,8)	2 (1,3)*

Tabla 21. Mutaciones halladas en pacientes obesos y con comportamiento por atracón. HGMD, Human Gene Mutation Database; AA, aminoácidos; N, número de alelos identificados *p < 0.05 obesos vs pacientes.

Los pesos (63,2 y 68,4 Kg) e IMC (23,5 y 25,4 kg/m^2) de los pacientes con BN portadores de la variante 251L se compararon con el peso (61,33 ±17,91 kg) e IMC (23,41 ± 6,20 kg/m^2) medio de los pacientes no portadores y no se encontraron diferencias estadísticamente significativas. Estos dos pacientes mostraron mayores puntaciones que los no portadores en varias escalas: puntación total de EDI-2 (153.0 ± 48.1 vs. 116.6 ± 38.1), *Bulimia* (15.0 ± 7.1 vs. 10.0 ± 7.2) y *Conciencia Interoceptiva*

(18.0 ± 2.8 vs. 14.1 ± 6.7). De la misma manera se obtuvieron valores más elevados en la escala GSI (2.25 ± 0.64 vs. 1.93 ± 0.84) del test SCL-90R. Sin embargo las diferencias no fueron estadísticamente significativas para ninguna de las categorías medidas.

A continuación, se resumen los resultados obtenidos del gen *MC4R*

- La frecuencia total de las variantes *MC4R* identificadas en el grupo de obesos fue del 3,8%.
- Todos presentaban las mutaciones en heterocigosis, excepto un sujeto heterocigoto compuesto portador de dos polimorfismos (S127L y G252S).
- En el grupo de comportamiento de atracones, se identificaron sólo dos pacientes de BN portadoras del polimorfismo I251L.
- La frecuencia total de polimorfismos en el gen *MC4R* del grupo de BN con comportamiento de atracones fue significativamente menor que la del grupo de obesos.

6. DISCUSIÓN

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En los últimos años se han publicado excelentes revisiones que proporcionan una visión en profundidad acerca de los polimorfismos genéticos implicados en las rutas biológicas relacionadas con la conducta alimentaria (Monteleone y Maj, 2008; Rask-Andersen y cols., 2010; Scherag y cols., 2010; Hinney y Volckmar, 2013; Trace y cols., 2013). Sin embargo, estos mismos autores están de acuerdo en que es llamativo que la mayoría de los genes conocidos implicados en la regulación central de peso todavía no se han explorado en estudios de asociación con TCA (Rask-Andersen y cols., 2010; Trace y cols., 2013). De hecho, se han estudiado menos del 40% de los más de 100 genes que participan en estos procesos (Olszewski y cols., 2008; Rask-Andersen y cols., 2010).

A este respecto, Day y cols. (Day y cols., 2009) reflexionan acerca de los vínculos entre la obesidad y los TCA y de cómo la obesidad se considera generalmente una enfermedad con orígenes metabólicos y genéticos, mientras que los TCA se han considerado tradicionalmente como síndromes ligados a la cultura occidental con tratamiento basado, principalmente, en intervenciones psicológicas. Los autores argumentan en contra de esta polarización y hacen comentarios sobre las similitudes en el fenotipo, las dimensiones de la personalidad y los factores de riesgo. Entre las similitudes entre ambos trastornos se pueden citar la insatisfacción corporal, la baja autoestima, la ansiedad y depresión, el abuso de sustancias, las dietas, los atracones, una historia de abuso sexual y/o físico, etc. (Day y cols., 2009). Mientras que algunos de estos rasgos son el resultado evidente de la enfermedad, es probable que la dirección de la causalidad vaya en ambos sentidos. De hecho, se ha demostrado que los síntomas psicopatológicos que alteran la conducta alimentaria conducen a su vez a la obesidad (Lee, 2007).

También podemos hablar de los vínculos entre los TCA y la obesidad desde la perspectiva de la genética evolutiva. Sin duda, el estilo de vida occidental favorece el desarrollo de la obesidad y de los TCA, sin embargo existen otros factores que claramente contribuyen al desarrollo de estas patologías. ¿Podría la selección natural haber favorecido la propagación de genes que predisponen a estos dos trastornos? Entre las varias hipótesis evolutivas que se han propuesto, es interesante que tanto el punto de vista adaptativo de la obesidad propuesto por Speakman (Speakman, 2013), como la hipótesis *Adaptación para huir de la hambruna* de la AN propuesta por Guisinger (Guisinger, 2003) sugieren que la necesidad de sobrevivir a ambientes hostiles podría haber inducido cambios en genes que dieran lugar o bien a la acumulación de grasa, o a la restricción en la ingesta alimentos. Estas hipótesis evolutivas sostienen que estos cambios podrían ser el origen de los mecanismos evolutivos que predisponen a la obesidad y a los TCA. Además, existen otras evidencias que apoyarían la existencia de una conexión genética entre estos dos trastornos. Bouchard y cols. publicaron la existencia de un pico de ligamiento común en el cromosoma 15 para la susceptibilidad a los TCA y a la obesidad (Bouchard y cols., 2004). Del mismo modo, mediante análisis de ligamiento del genoma completo se han encontrado regiones en el cromosoma 10 que poseen picos de ligamiento comunes para el IMC y obesidad con BN (Bulik y cols., 2003; Kim y cols., 2013).

Siguiendo nuestra hipótesis de trabajo que establece la existencia de una conexión íntima entre los TCA y la obesidad, podríamos esperar que las alteraciones genéticas en las vías centrales que participan en la regulación de la conducta alimentaria y que pueden desembocar en obesidad, también puedan favorecer el desarrollo de patrones de conducta aberrantes que favorezcan la instauración de un TCA.

En el presente estudio, nos hemos centrado en tres genes con expresión en el sistema nervioso central, cuya variabilidad genética se ha asociado consistentemente con el IMC o la obesidad.

Con respecto al primer gen de estudio, numerosos trabajos señalan que *BDNF* desempeña un papel fundamental en el control de la conducta alimentaria, peso corporal y homeostasis energética, así como en el deterioro cognitivo (Kernie y cols., 2000; Gray y cols., 2006; Burns y cols., 2010; Cordeira y Rios, 2011; Rios, 2011; Rosas-Vargas y cols., 2011). Estas evidencias han propiciado que el *BDNF* sea el gen asociado con obesidad más estudiado en el campo de los TCA.

Nuestros análisis no detectaron asociaciones significativas entre los SNP del gen *BDNF* analizados y un mayor riesgo de padecer AN o BN. Este hallazgo es consistente con el único estudio de asociación del genoma completo (GWAS) realizado en pacientes con AN (Pinheiro y cols., 2010). La mayoría de la información disponible en relación a los estudios de asociación genética es referente a los polimorfismos Val66Met y C-270T. El primero de ellos se ha asociado con un mayor riesgo de padecer estos trastornos, particularmente con AN de tipo restrictivo (Ribases y cols., 2004; Monteleone y cols., 2006; Dmitrzak-Weglarz y cols., 2007; Gratacos y cols., 2007). Sin embargo, otros grupos de investigación han obtenido resultados negativos, acordes a los obtenidos en esta tesis (de Krom y cols., 2005; Friedel y cols., 2005; Dardennes y cols., 2007; Rybakowski y cols., 2007; Ando y cols., 2012). Además, en nuestra población, el análisis del subgrupo de pacientes con ANR (datos no mostrados) no reveló asociaciones más significativas que las obtenidas con el global de la población anoréxica. De la misma manera, y en consonancia con estudios anteriores (Friedel y cols., 2005; Dmitrzak-Weglarz y cols., 2007), no detectamos ninguna asociación entre el riesgo de padecer TCA y el SNP C-270T. Hong y cols. han evaluado recientemente, la controversia acerca de la influencia de las variantes

genéticas del *BDNF* y sugieren que la correcta evaluación del fenotipo puede estar detrás de los resultados contradictorios publicados (Hong y cols., 2011).

Los análisis de haplotipo del gen *BDNF* podrían ayudar a explicar estas inconsistencias, sin embargo, todavía son pocos los estudios de este tipo realizados en el ámbito de los TCA. Tan sólo un estudio de asociación familiar ha identificado un haplotipo compuesto por dos SNPs asociado con AN (Mercader y cols., 2007). En nuestro trabajo no hemos podido confirmar la presencia de ningún haplotipo que confiera mayor riesgo de padecer un TCA.

A continuación valoramos el efecto de la variabilidad genética del *BDNF* en los parámetros antropométricos de las pacientes. Los resultados revelaron que el genotipo mutante TT del SNP rs16917237 estaba asociado con mayor peso e IMC en sujetos con BN. No hay evidencias previas que relacionen este SNP con el peso corporal en pacientes con TCA. Sin embargo, el consorcio GIANT (Genetic Investigation of Anthropometric Traits (*htttp://www.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium*) ha incluido recientemente este polimorfismo en una lista de posibles marcadores de obesidad. En este sentido cabe mencionar que los pacientes con BN tienden a mostrar mayor IMC que los controles y mayores tasas de obesidad pre-mórbida (Fairburn y cols., 1997; Fairburn y cols., 2003). Por otro lado, un estudio GWAS realizado en sujetos sanos, ha evidenciado la asociación del SNP rs10767644 con mayor IMC (Speliotes y cols., 2010). Aunque en nuestro trabajo no hemos probado el efecto de esta variante, sí evaluamos el SNP Val66Met, que se encuentra en fuerte desequilibrio de ligamiento con el SNP rs10767664 (r^2 >0.75), obteniéndose resultados negativos en relación al IMC. Otros trabajos que evalúan el efecto del SNP Val66Met en los valores antropométricos coinciden con nuestros resultados (Ribases y cols., 2004; Ribases y cols., 2005). Por tanto, serían necesarios estudios más amplios en el ámbito de los TCA, para confirmar la asociación observada por el estudio GWAS en la población

general. Por otra parte, Ribasés y cols. han mostrado que las pacientes con BN (sin historia anterior de AN) portadoras del alelo -270T presentaban un aumento en el peso corporal (Ribases y cols., 2004; Ribases y cols., 2005), asociación que no observamos en nuestro estudio. Esta discrepancia podría reflejar la heterogeneidad fenotípica de nuestra muestra bulímica, que incluía a mujeres con un historial previo de AN, en el curso de la cual a menudo, aparecen síntomas bulímicos y/o se produce un cambio al síndrome completo de BN (Tozzi y cols., 2005).

En general no está claro cómo las variantes del *BDNF* pueden modular el peso corporal. Una posible hipótesis sería la presentada por Saito y cols. que identificaron una correlación positiva entre los niveles de BDNF en suero y el IMC en mujeres con BN (Saito y cols., 2009). Aunque el efecto bioquímico exacto del SNP rs16917237, asociado con mayor peso corporal en nuestra población bulímica, es hoy en día desconocido, el hecho que otros SNPs intrónicos puedan afectar a los niveles circulantes de proteína en pacientes con BN (Saito y cols., 2009; Terracciano y cols., 2011) podría sugerir la existencia de un mecanismo subyacente en la regulación positiva del peso e IMC.

El principal objetivo de esta parte del trabajo fue investigar si la variabilidad genética del *BDNF* se correlacionaba con las dimensiones de personalidad y/o con los síntomas psicopatológicos que presentan las pacientes con TCA. Hasta donde sabemos, este tipo de estudio sólo ha sido abordado en un trabajo previo en sujetos polacos con AN, en el cual se analizaron los SNPs Val66Met y C-270T (Rybakowski y cols., 2007). Los autores concluyeron que el alelo -270T estaba relacionado con ciertos rasgos de la personalidad. En nuestro grupo de pacientes con AN, fue el alelo -270C el que se correlacionaba con la *Desconfianza Interpersonal*, es decir, la reticencia a formar relaciones personales íntimas. Los resultados contradictorios observados en ambos trabajos podrían estar influenciados por la utilización de diferentes cuestionarios para la evaluación de los pacientes, el diferente origen étnico

o la distinta configuración de los estudios (pacientes ambulatorios frente a pacientes hospitalizados).

El enfoque de ventana deslizante (del inglés *sliding-window approach*) aplicado en este análisis reveló que uno de los cuatro loci resultantes en el análisis tenía un amplio efecto sobre las puntuaciones de las escalas del cuestionario SCL-90R. Los síntomas psicopatológicos que se miden mediante este cuestionario no están íntimamente relacionados con los TCA, sino que son comunes a diversos trastornos psiquiátricos. Por tanto, la asociación de ciertos haplotipos del *BDNF* con estos síntomas, el insignificante efecto en los patrones medidos por el cuestionario EDI-2, específico de los TCA, y el hecho de que estos haplotipos no influyeran en la susceptibilidad a estos trastornos, todo ello sugiere que la variabilidad genética del *BDNF* puede ser importante para el desarrollo de determinados problemas psicopatológicos que podrían ser el reflejo de los trastornos comórbidos que presentan los pacientes con TCA.

Ahora bien ¿por qué las variaciones genéticas en el gen *BDNF* podrían desembocar en síntomas psicopatológicos en pacientes con TCA? Existen pruebas convincentes que sugieren que la fisiopatología de varias enfermedades mentales podría ser el resultado de una alteración en la plasticidad sináptica causada por una expresión y liberación alterada de BDNF (Lang y cols., 2004; Duman y Monteggia, 2006; Sen y cols., 2008). Además, Mercader y cols. (Mercader y cols., 2007) demostraron que ciertos haplotipos del gen *BDNF* son capaces de modular en pacientes con BN las concentraciones de la proteína que, a su vez, se correlacionan negativamente con las puntuaciones de los cuestionarios de evaluación psicológica en pacientes con TCA (Mercader y cols., 2007; Saito y cols., 2009; Mercader y cols., 2010). Por lo tanto, se podría especular que los niveles alterados de BDNF como resultado de la presencia de ciertos haplotipos en nuestros pacientes, podrían haber contribuido al desarrollo de los rasgos psicopatológicos observados. Un hecho que

apoya esta hipótesis es que las mismas escalas del SCL-90R que se correlacionaban con los niveles plasmáticos de BDNF en el estudio de Mercader y cols. (Mercader y cols., 2007) fueron las afectadas por el locus identificado en nuestro estudio.

Nuestros resultados en este primer estudio sugieren que la variabilidad en el locus del gen *BDNF* puede afectar no solo a las características antropométricas, sino también a los síntomas psicopatológicos de los pacientes con TCA. Probablemente las alteraciones psicopatológicas son más indicativas de los trastornos comórbidos asociados a los TCA.

El siguiente de los estudios se centró en el papel del gen *NEGR1*. Las alteraciones genéticas que se producen en el complejo sistema neuronal destinado a mantener el correcto equilibrio energético mediante la ingesta de alimentos son factores conocidos que contribuyen al desarrollo y mantenimiento de la obesidad. En este sentido, las variantes del gen *NEGR1*, cuyos loci se han asociado en repetidas ocasiones con un riesgo incrementado de padecer obesidad (Renstrom y cols., 2009; Thorleifsson y cols., 2009; Willer y cols., 2009; Zhao y cols., 2009; Speliotes y cols., 2010; Magi y cols., 2013; Mejia-Benitez y cols., 2013; Wheeler y cols., 2013), son unos posibles candidatos de estudio en el ámbito de los TCA.

El principal hallazgo de esta parte del estudio fue que la variabilidad genética en una región del locus del gen *NEGR1* presentaba un impacto muy significativo en las puntuaciones obtenidas por los pacientes bulímicos en varias dimensiones de la personalidad. Es de destacar que tanto el estudio de SNPs individuales como los dos estudios de haplotipo señalaron a la misma región como un locus con influencia en los rasgos psicológicos relacionados con los TCA.

Los mecanismos que están detrás de los comportamientos que presentan los pacientes con trastornos psiquiátricos son extremadamente complejos, pero se sabe

que algunos de estos mecanismos pueden estar influidos por la contribución de determinados genes (Cloninger, 1999; Ebstein y cols., 2000). En este sentido, el rasgo más afectado por la variabilidad genética del *NEGR1* en los pacientes con BN fue la *Conciencia Interoceptiva*, que mide la capacidad del individuo para discriminar entre las emociones y sentimientos y, lo más interesante, entre las sensaciones de hambre y saciedad (Garner, 1991). Se ha sugerido que el déficit en *Interoceptividad* puede ser un factor que sirva de puente entre el funcionamiento anormal de las redes neuronales y la presentación de los síntomas característicos de la BN, como los atracones y las purgas. De esta manera, el sistema de regulación interno tendría dificultad a la hora de interpretar de manera correcta las señales de hambre y saciedad, favoreciéndose la instauración de los rasgos bulímicos (Klabunde y cols., 2013). De hecho, varios estudios han demostrado que a las personas con BN les resulta difícil detectar determinadas señales corporales (Garfinkel y cols., 1978; Stein y cols., 2003; Zimmerli y cols., 2006). Esta hipótesis está apoyada por estudios de imagen que han identificado patrones de activación irregular en el cerebro cuando las mujeres con BN reciben un estímulo interoceptivo del gusto (Bohon y Stice, 2011). Siendo el *NEGR1* un gen con expresión principalmente cerebral con una destacada, aunque en parte desconocida, función en el crecimiento neuronal, proponemos que la variabilidad genética en su locus podría contribuir a alterar la sensibilidad interoceptiva. También es de destacar que el déficit en *Conciencia Interoceptiva* parece ser mayor en pacientes con BN que con AN u obesidad (Fassino y cols., 2004). Esto es consistente con la falta de asociación observada entre las variantes del *NEGR1* y este rasgo en nuestro grupo de pacientes anoréxicas, y con el hecho de que las pacientes con BN en nuestra serie muestran puntuaciones totales del test EDI-2 más altas que las mujeres con AN.

Curiosamente, Klabunde y cols. (Klabunde y cols., 2013) determinaron que el déficit de procesamiento interoceptivo también estaba presente en mujeres que se

recuperaron de BN. Los autores plantean la cuestión de si este déficit podría ser una consecuencia de haber sufrido BN o si, por el contrario, es un rasgo biológico que está presente antes del desarrollo de los síntomas bulímicos. Nuestros resultados describen una alteración genética como una posible fuente de error a la hora de procesar correctamente las sensaciones de hambre y saciedad, lo que apoyaría la segunda teoría.

La *Ineficacia*, que evalúa los sentimientos de incapacidad general, inseguridad, vacío, auto desprecio y falta de control sobre la propia vida, fue otra de las escala del cuestionario EDI-2 más afectada por la variabilidad genética del *NEGR1* en los pacientes con BN. El concepto de ineficacia o falta de autoestima, como un factor de riesgo para los TCA es aún controvertido (Dobmeyer y Stein, 2003; Combs y cols., 2010), pero se ha sugerido que este rasgo podría estar mediado por otros factores de riesgo para la BN, como por ejemplo el abuso infantil (Akkermann y cols., 2012; Groleau y cols., 2012); una información que no estaba disponible en nuestro estudio. Goethals y cols (Goethals y cols., 2007) han demostrado que puntuaciones patológicas en la escala de *Ineficacia* están apoyadas por procesos biológicos en el cerebro. Por ejemplo, las puntaciones correlacionaban con el flujo sanguíneo en ciertas regiones cerebrales. La falta de conocimiento acerca de la función específica que desempeña el gen *NEGR1* y el mecanismo a través del cual este gen puede afectar al comportamiento, dificulta la tarea de establecer una conexión entre la variabilidad en el locus del gen y la *Ineficacia*. Sin embargo, se ha publicado que este rasgo, así como la *Obsesión por la delgadez*, escala que también se ha visto afectada por la variabilidad del gen *NEGR1* en nuestra muestra, se correlacionaban con variantes genéticas de otros genes de expresión cerebral (Frieling y cols., 2006; Nisoli y cols., 2007; Mikolajczyk y cols., 2010). Además, en línea con nuestros resultados, se ha comprobado que las relaciones entre los genotipos y estas escalas son más explícitas en pacientes con BN que en mujeres con AN (Mikolajczyk y cols., 2010).

Por otro lado, nuestros datos no demostraron que existiese un riesgo aumentado de padecer BN o AN asociado a ningún SNP o haplotipo, lo que sugiere que los rasgos de personalidad afectados podrían ser más importantes en términos de severidad o duración del trastorno que en términos de mayor susceptibilidad. Del mismo modo, no hemos observado ningún efecto de las variantes del gen *NEGR1* sobre el peso o el IMC en nuestro grupo de pacientes bulímicas. Sería muy complicado demostrar una asociación de esos SNPs con una tendencia a la obesidad en pacientes bulímicos, ya que su posible impacto sobre la obesidad sería probablemente anulado por mecanismos compensatorios como purgas o actividad física extrema.

Es importante mencionar, que aunque el gen *NEGR1* se ha asociado en repetidas ocasiones con cambios que conducen a obesidad, lo que constituye la columna vertebral de nuestra hipótesis, algunos estudios no han obtenido resultados positivos a este respecto (Ng y cols., 2010; Leon-Mimila y cols., 2013). Del mismo modo, no podemos descartar que las variantes del gen *NEGR1* sean en realidad el reflejo de un amplio rango de asociaciones con otros genes. Recientemente, se ha demostrado que el mayor riesgo de padecer obesidad que estaba asociado a una deleción en el gen *NEGR1*, estaba en realidad asociado a otra deleción de 8 kb en una región adyacente que abarca el sitio de unión del factor de transcripción NKX6.1, implicado en el desarrollo neuronal (Hafler y cols., 2008).

Nuestros resultados indican que el gen *NEGR1* podría ser un locus importante que influye en ciertas dimensiones de la personalidad, en concreto en la *Conciencia Interoceptiva, Ineficacia* y *Obsesión por la Delgadez*, en pacientes con BN. Dentro de la ya comentada falta de estudios que hay respecto al *NEGR1* y TCA, sí que hay ciertos indicios que apoyan nuestras conclusiones. Así, Maccarrone y cols han demostrado que los niveles de proteína NEGR1 están relacionados con la depresión y

otros trastornos psiquiátricos que también pueden presentar los pacientes con TCA (Maccarrone y cols., 2013).

El último de los estudios incluidos en esta tesis presentó un diseño diferente al de los dos anteriores (ver sección de *Metodología*) y se centró en el papel del *MC4R*. Como ya se ha comentado, éste es un gen de expresión hipotalámica que participa en el proceso de señalización mediado por la α-MSH (Mountjoy y cols., 1994). Numerosos estudios han relacionado la variabilidad en el gen con la obesidad, de hecho, más del 4% de los casos de obesidad mórbida son atribuibles a mutaciones en *MC4R* (Vaisse y cols., 2000; Farooqi y O'Rahilly, 2006).

En este tercer estudio identificamos mediante secuenciación directa 10 variantes diferentes en el gen *MC4R* en 12 individuos obesos de inicio precoz, siendo la frecuencia global de mutación en estos pacientes del 3,8 %, similar a la obtenida en estudios previos (Farooqi y cols., 2003; Lubrano-Berthelier y cols., 2006). Por otro lado, tan solo dos pacientes no obesas con comportamiento de atracón y purga eran portadoras de la misma mutación (I251L) en el gen.

La mayoría de los SNPs identificados en nuestro estudio han sido objeto de estudios funcionales. De hecho, algunas de las mutaciones producen una respuesta deficiente de MC4R en respuesta a α-MSH, lo que sugiere la existencia de un mecanismo subyacente que favorece el desarrollo de obesidad (Hinney y cols., 1999; Lubrano-Berthelier y cols., 2003; Lubrano-Berthelier y cols., 2003). En nuestro estudio, todos los pacientes obesos excepto uno eran portadores de una mutación en heterocigosis. La excepción fue un individuo que era portador de dos mutaciones (S127L y G252S), aunque tan solo el SNP S127L afectaría a la función de la proteína según Hinney y cols (Hinney y cols., 1999). Los polimorfismos R147G y G323E (Albuquerque y cols., 2014), identificados en tres pacientes obesos en nuestra

muestra, causan una sustitución en un aminoácido. Según el software de predicción *Polyphen-2*, el efecto del SNP R147G sería deletéreo, mientras que el impacto del G323E en la proteína sería leve y por tanto su contribución al fenotipo obeso es dudosa (Albuquerque y cols., 2014). A la misma conclusión se llega si se utiliza el software de predicción *SIFT* (Puntuación del SNP R147 = 0, dañino; puntuación del SNP G323= 0.15, tolerado).

La localización hipotalámica de la proteína codificada por gen *MC4R* unida al hecho de que alteraciones en su locus pueden causar hiperfagia (Huszar y cols., 1997), hace que sea posible especular que los portadores de mutaciones presenten determinadas características psicopatológicas. Para analizar esta hipótesis, las 97 pacientes con TCA incluidas en el estudio realizaron los cuestionarios EDI-2 y SCL-90R, que en estudios previos se han utilizado para identificar síntomas psicopatológicos en pacientes con episodios de purga y atracón (Brambilla y cols., 2009). Las dos pacientes portadoras del polimorfismo I251L mostraron mayores puntuaciones en el valor total del cuestionario EDI-2 y en dos escalas que podrían estar relacionadas con la actividad del gen *MC4R*: *Bulimia,* que mide los episodios de atracón y purga y *Conciencia Interoceptiva,* que evalúa la capacidad de discriminación entre las sensaciones de hambre y saciedad. Del mismo modo, estas dos pacientes presentaron valores más elevados en los resultados totales del cuestionario SCL-90R y en las escalas de *Ansiedad* y *Obsesivo-Compulsivo* del mismo. Sin embargo, existen varias razones para creer que estos hallazgos no son clínicamente relevantes. En primer lugar, las diferencias no son estadísticamente significativas para ninguno de los resultados obtenidos (aunque es obvio que la presencia de tan solo dos portadoras limita el poder estadístico del estudio). En segundo lugar, las dos pacientes también presentaron mayores puntuaciones en otras escalas psicopatológicas que no se asocian directamente con el comportamiento de atracón (resultados no mostrados). En tercer lugar, y más importante, se podrían relacionar los valores elevados en las

escalas *Ansiedad* y *Obsesivo-Compulsivo* con la incapacidad de reconocer las sensaciones de saciedad como consecuencia de la presencia de un gen *MC4R* defectuoso. Sin embargo, para llegar a esta conclusión tendríamos que considerar las consecuencias funcionales del SNP I251L, un tema que ha generado una gran controversia. Por ejemplo, Branson y cols. (Branson y cols., 2003) identificaron está mutación en cinco pacientes obesos con trastorno por atracón y mostraron una fuerte asociación entre este trastorno y otras variantes genéticas del gen *MC4R.* Sin embargo, estudios posteriores no han reproducido estos resultados y han concluido que los episodios de atracón y purga no son característicos de individuos obesos adultos portadores de mutaciones en el gen *MC4R* (Hebebrand y cols., 2004; Lubrano-Berthelier y cols., 2006; Valette y cols., 2013). Las investigaciones *in vitro* establecen que el SNP I251L no parece afectar a la función del receptor (Vaisse y cols., 2000; Nowacka-Woszuk y cols., 2011; Thearle y cols., 2012), lo que apoyaría la hipótesis de que no existe asociación entre la mutación y los episodios de purga y atracón.

En resumen, los resultados de nuestro tercer trabajo establecen que las variantes del gen *MC4R* son más frecuentes en individuos obesos que en pacientes no obesos con episodios de atracón y purga. Confirmamos la ausencia de asociación entre las variantes del gen *MC4R* y el comportamiento de atracón y purga, en pacientes no obesas diagnosticadas de TCA, lo que ya estaba descrito en pacientes obesas.

Existen una serie de limitaciones en esta tesis que deben tenerse en cuenta. En primer lugar, el tamaño de nuestra muestra es limitado, particularmente el del grupo de pacientes con BN, lo que podría limitar la reproducibilidad de los resultados. Por otro lado esto ha permitido que todos los pacientes hayan sido diagnosticados y tratados por el mismo equipo clínico y en las mismas condiciones a lo largo de un periodo corto de tiempo, favoreciendo de esta manera la homogeneidad de la muestra. En segundo lugar, no hemos considerado las distintas categorías clínicas de los TCA y

las diferentes escalas de evaluación psicológica a la hora de corregir estadísticamente por comparaciones múltiples, ya que se ha sugerido en estudios similares (Mercader y cols., 2007) que este procedimiento es demasiado estricto para detectar correlaciones moderadas con varios rasgos psicológicos. En tercer lugar, nuestra muestra control para el estudio del gen *BDNF* y *NEGR1* estaba formada por estudiantes universitarios, lo que podría constituir un sesgo de selección en términos de diferencias socioeconómicas entre los grupos de control y pacientes. En cuanto a la muestra control del estudio del gen *MC4R*, no se incluyeron individuos con peso normal. Finalmente, hay que tener en cuenta que en el análisis del gen *NEGR1* no pudimos determinar tres de los tag-SNPs seleccionados inicialmente, y por tanto, no se pudo evaluar la relevancia de las regiones cubiertas por estas variantes.

7. CONCLUSIONES

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Basándonos en los resultados del presente trabajo, podemos extraer las siguientes conclusiones:

1. Ninguno de los SNP o haplotipos analizados del gen *BDNF* desempeña un papel relevante en el riesgo de padecer AN o BN.
2. El genotipo TT del SNP rs16917237 se asocia significativamente con un aumento del peso y del índice de masa corporal en pacientes bulímicas.
3. Los haplotipos CGC y CGT del locus 3 (rs10835210 / rs16917237 / C-270T) en el gen *BDNF* tienen un efecto muy marcado sobre las puntuaciones del cuestionario SCL-90R, tanto en pacientes con anorexia nerviosa como con bulimia nerviosa. Sin embargo, los resultados del cuestionario EDI-2 no se ven modificados por la presencia de estos haplotipos.
4. Ninguno de los SNPs o haplotipos analizados del gen *NEGR1* se asocian con un mayor riesgo de padecer un TCA ni con parámetros antropométricos.
5. En pacientes con BN, cuatro SNPs de *NEGR1* (rs12740031, rs10789322, rs6659202 y rs591540) se correlacionan con las puntuaciones de las escalas *Obsesión por la Delgadez*, *Ineficacia* y *Conciencia Interoceptiva.*
6. Los SNPs de *NEGR1,* rs12740031, rs10789322, rs954299 y rs2422021, forman un bloque haplotípico relacionado con las puntuaciones totales del cuestionario EDI-2 en pacientes con BN. Este bloque está incluido en una región central del gen asociada a su vez con mayores puntaciones de las escalas *Obsesión por la delgadez*, *Ineficacia*, *Conciencia Interoceptiva* y *Bulimia* en pacientes con BN.
7. La frecuencia de mutaciones en el gen *MC4R* es mayor en pacientes obesos que en pacientes con episodios de purga y atracón con peso normal.

8. Estas pacientes portadoras de variantes *MC4R* no presentan valores de peso, IMC o características psicopatológicas diferentes de las pacientes no portadoras.

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9. PUBLICACIONES

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ORIGINAL CONTRIBUTION

BDNF genetic variability modulates psychopathological symptoms in patients with eating disorders

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Abstract The brain-derived neurotrophic factor (*BDNF*) gene may influence eating behavior, body weight and cognitive impairments. We aimed to investigate whether *BDNF* genetic variability may affect anthropometric and psychological parameters in patients with anorexia or bulimia nervosa (AN, BN) and/or modulate the risk for the disorder. A total of 169 unrelated female patients and 312 healthy controls were genotyped for two common *BDNF* single-nucleotide polymorphisms (SNPs), Val66Met and C-270T, and several selected tag-SNPs. Associated personality characteristics and psychopathological symptoms were assessed by the EDI-2 and SCL-90R inventories, respectively. No single SNP or haplotype played a relevant role in the risk for AN or BN. The rs16917237 TT genotype was significantly associated with increased weight (74.63 ± 16.58 vs. 57.93 ± 13.02) and body mass index (28.94 ± 6.22 vs. 22.23 ± 4.77) in the BN group after correcting for multiple testing. Haplotype analyses using a sliding window approach with three adjacent SNPs produced four loci of interest. Locus 3 (rs10835210/rs16917237/C-270T) showed a broad impact on the measured psychopathological symptoms. Haplotypes CGC and CGT in this locus correlated with scores in all three scales of the SCL-90R inventory, both in AN and BN patients. In contrast, the results of the EDI-2 inventory were largely unaffected. These preliminary results suggest that variability in the *BDNF* gene locus may contribute to anthropometric characteristics and also psychopathological symptoms that are common but not exclusive of ED patients.

Keywords BDNF · Genetic polymorphism · Anorexia nervosa · Bulimia nervosa · Eating disorder

Introduction

Anorexia nervosa (AN) and bulimia nervosa (BN) are severe eating disorders (ED) affecting mainly young females. They are characterized by alterations in eating behavior and weight regulation driven by low self-esteem due to weight preoccupation and perceptions toward body weight and shape. The etiology of these disorders is complex and it is widely accepted that biological, psychosocial and genetic factors are involved [1]. A number of candidate genes with a role in the regulation of eating behavior and body weight have been considered in studies with ED patients [1–3]. One of them is the brain-derived neurotrophic factor (*BDNF*), which is widely expressed in the brain and is involved in neuronal development and synaptic plasticity. Several lines of evidence indicate that this gene plays a role in energy balance and reward centers of the brain, thus impacting eating behavior, body weight and cognitive impairments in ED [4–7].

Two single-nucleotide polymorphisms (SNPs) in the *BDNF* gene, namely a valine to methionine substitution in

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residue 66 (rs6265) and a C-270T transition in the 5′-untranslated (UTR) region (rs56164415), have been the focus of most studies in the ED setting. These works have mostly addressed the role of these variants in the susceptibility to the disorder. For instance, an association between the Met66 allele and the risk for AN, particularly the restricting subtype, has been reported [8, 9], albeit contradictory data have recently been published [10, 11]. In addition, the -270C allele has been shown to modulate the risk for BN and the age at onset of weight loss in ED patients [9].

Previous reports in healthy subjects suggest an association between *BDNF* variant alleles and personality dimensions such as neuroticism [12], introversion [13] and various anxiety- and depression-related traits [14]. However, the role of *BDNF* genetic variants as modulators of personality characteristics and psychopathological symptoms in ED patients has been largely overlooked. Individuals with ED display characteristic personality profiles of several traits each of which has been shown to be at least moderately heritable [15]. Studies on personality dimensions have revealed differences between ED patients and healthy females [16, 17], and high levels of traits such as depression, anxiety, impulsiveness, drive for thinness, etc. have been associated with the severity of AN and BN [18, 19].

In the present study, we have aimed to investigate whether *BDNF* genetic variability may influence personality characteristics and psychopathological symptoms in ED patients and/or modulate the risk for the disorder. For this, in a population of patients with AN or BN and healthy subjects, we have analyzed the presence of the two most commonly studied *BDNF* SNPs, namely Val66Met and C-270T, as well as that of four other tag-SNPs located in the gene (rs11030102, rs16917237, rs11030119 and rs10835210), which have previously been suggested to impact different psychiatric disorders and/or serum levels of BDNF [20–23].

Methods

Subjects

The study group consisted of 169 unrelated consecutive female patients with AN ($n = 106$) or BN ($n = 63$), some of whom had also participated in two other recent studies by our group [24, 25]. The patients attended the collaborating Eating Disorders Unit at the Mental Health Outpatient Clinic in Badajoz (Spain), and were diagnosed by one psychiatrist and one psychologist using the ED section of the Structured Clinical Interview for DSM-IV [26]. Anthropometric (weight, height and body mass index) and psychological parameters (see below) were then collected. Diagnosis was blind to genotype. Exclusion criteria, determined upon screening, included dementia, mental retardation, schizophrenia, Turner's syndrome, other neurological disorders and underlying endocrine pathologies.

A total of 312 healthy women from the same geographical area as the patients (Health District of Badajoz, Spain) were recruited among University students and staff. Interviews were carried out to guarantee that they had never been diagnosed as having any psychiatric disorder or received any psychiatric treatment. None of the participating control subjects showed anthropometric parameters indicative of present ED.

All the participants were white Spanish individuals who gave written informed consent. The study protocol was approved by the Ethics Committee of the University of Extremadura and was conducted in accordance with the Declaration of Helsinki and its subsequent revisions.

Table 1 Single-nucleotide polymorphisms genotyped in the *BDNF* genomic region

SNP reference	Chromosome position[a]	Transcript position	Alleles	MAF
rs6265	27619916	Coding	G/A (Val66Met)	0.21
rs11030102	27621596	Intronic	C/G	0.18
rs10835210	27635910	Intronic	A/C	0.44
rs16917237	27642383	Intronic	G/T	0.24
rs56164415 (C-270T)	27661737	Intronic	C/T	0.10
rs11030119	27668102	Intronic	A/G	0.24

MAF minor allele frequency in the control population

[a] NCBI reference sequence NT_009237.18

SNPs selection

European population (CEU) SNP data were downloaded from the International Haplotype Mapping Project web site (http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap27_B36). To capture the common SNP variation in the *BDNF* gene, we analyzed the coding sequence and adjacent 3′ and 5′UTR regions and selected four tag-SNPs (rs11030102, rs16917237, rs11030119 and rs10835210) using the *Tagger* function of the Haploview 4.0 software (Table 1). The SNPs were selected with a minor allele frequency of at least 0.1, a pair-wise tagging r^2 value of at least 0.80 and were located within a 46.5 kb region that encompasses the *BDNF* gene (contig NT_009237.18; chromosome positions 27681596 to 27728102). All the SNPs were intronic and captured most of the common variations described for the gene and adjacent regions in public databases.

Genotype analysis

Blood samples from all participants were stored at −80 °C until analysis. Genomic DNA was isolated from peripheral blood leukocytes in 2-ml aliquots of whole-blood samples with a Qiagen blood midi kit (Qiagen Inc., Chatsworth, CA, USA). The purified DNA samples were then stored at 4 °C in sterile plastic vials.

Genotype analyses for rs11030102, rs16917237, rs11030119 and rs10835210 were performed with the single-base extension polymerase chain reaction Sequenom iPLEX-Gold and the mass spectrometry-based platform MassARRAY MALDI-TOF at the Spanish Genotyping National Centre (CEGEN-ISCIII). In brief, the analyses consisted of an initial locus-specific PCR, followed by single-base extension using mass-modified dideoxynucleotide terminators of an oligonucleotide primer which anneals immediately upstream of the polymorphic site of interest. Using MALDI-TOF mass spectrometry, the distinct mass of the extended primer identifies the allele [27].

PCR-RFLP (restriction fragment length polymorphisms) techniques were utilized for the determination of the Val66Met and C-270T SNPs as described elsewhere [9]. The analysis of samples with unclear restriction patterns was confirmed by direct sequencing (Applied Biosystems, Foster City, CA, USA).

Psychometric evaluation

The examination of personality characteristics and psychopathological symptoms in ED patients was performed on the day of their first visit to the Eating Disorders Unit by two self-reported questionnaires, namely the Eating Disorders Inventory Test-2 (EDI-2) and the Symptom Checklist 90 Revised (SCL-90R).

The EDI-2 is a 64-item questionnaire designed to assess the cognitive and behavioral features characteristic of ED patients [28]. It is composed of eight main subscales (Drive for Thinness, Bulimia, Body Dissatisfaction, Ineffectiveness, Perfectionism, Interpersonal Distrust, Interoceptive Awareness and Maturity Fears) and three additional ones (Asceticism, Impulse Regulation and Social Insecurity). This inventory has been adapted for the Spanish population showing high internal consistency between the different subscales [29]. The SCL-90R is a 90-item psychiatric self-reported inventory that evaluates a broad range of psychological problems and symptoms of psychopathology through three Global Indexes (Global Severity Index, GSI; Positive Symptom Distress Index, PSDI; and Positive Symptom Total, PST) and nine primary symptom dimensions (somatization, depression, anxiety, hostility, phobic anxiety, paranoid ideation and psychoticism) [30]. This inventory has also been previously validated in the Spanish population [31].

Statistical analyses

The comparison of allelic and genotypic frequencies in cases and controls was performed with the χ^2 or Fisher's exact test as appropriate. The association between the analyzed polymorphisms and the risk to develop AN or BN was estimated by odds ratio (OR) with 95 % confidence interval (CI). The distribution of the quantitative variables across the different genotypes was compared by *T* test or ANOVA, as appropriate, and the results were corrected for multiple testing by the Bonferroni method, adjusting for the number of studied SNPs (significant p value <0.0083). To assess the association between *BDNF* variants and anthropometric parameters or personality characteristics and psychopathological symptoms, genotypes were considered as a dichotomous variable: value 0 was attributed to genotypes Val66Val, -270 CC; rs11030102 CC, rs126917237 GG, rs11030119 GG and rs10835210 CC as reference; and value 1 to heterozygous and mutant homozygous genotypes, assuming an effect for the presence of at least one allele at risk.

The statistical power of the sample size was evaluated with a log-additive genetic model, analyzing the frequency for carriers of the variant alleles with arbitrarily established effect size at 2.0 (type I error = 0.05). With the available sample size and minor allele frequencies ranging from 0.1 to 0.44, the statistical power for detecting associations with categorical variables in the AN and BN groups was 0.85–0.99 and 0.70–0.93, respectively (Quanto software v. 1.2.4, University of Southern California).

The SNPstats platform [32] was utilized to estimate general haplotype frequencies and to obtain linkage disequilibrium data. To refine the haplotype construction and to identify core regions with the potential to affect either the susceptibility for ED or the anthropometric/psychological parameters in the patients, we used a sliding window approach to construct successive and adjacent 3-SNP haplotypes. Subsequent case/control and quantitative associations were assessed using PLINK software v1.07 with a haplotype frequency cut-off of 0.1 [33].

In all instances, differences were considered to be significant when p values were under 0.05. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Table 2 shows descriptive and clinical characteristics of ED patients and healthy subjects, who displayed higher weight and BMI than AN patients ($p < 0.05$).

Table 2 Descriptive and clinical variables of patients with anorexia nervosa (AN) or bulimia nervosa (BN) and healthy controls

	AN	BN	Controls	*p* value
Age (years)	18.9 ± 6.2	20.9 ± 8.1	20.8 ± 7.6	NS
Height (m)	1.60 ± 0.07	1.61 ± 0.06	1.62 ± 0.07	NS
Weight (kg)	44.28 ± 6.08	59.16 ± 13.65	58.3 ± 10.12	<0.05[a]
BMI (kg/m^2)	17.19 ± 2.01	22.71 ± 5.03	21.99 ± 4.25	<0.05[a]
Age at onset (years)	16.9 ± 4.8	17.8 ± 4.9		
Total EDI-2 score	88.84 ± 45.53	117.27 ± 39.14		
GSI (SCL-90R) score	1.62 ± 0.85	1.96 ± 0.82		
PST (SCL-90R) score	63.33 ± 21.91	71.11 ± 17.07		
PSDI (SCL-90R) score	2.18 ± 0.64	2.4 ± 0.62		

Mean ± SD values are shown

[a] *p* value for the χ^2 comparison between controls and AN patients

Table 3 Genotype frequencies of the analyzed polymorphisms in patients with anorexia nervosa (AN) or bulimia nervosa (BN) and healthy controls

Polymorphism	Controls (%)	AN (%)	OR (CI)	*p* value	BN (%)	OR (95 % CI)	*p* value
rs11030102							
C/C	206 (66.2)	69 (65.1)	Reference		37 (58.7)	Reference	
C/G	96 (30.9)	31 (29.2)	0.96 (0.59–1.57)	0.90	24 (38.1)	1.39 (0.79–2.46)	0.3
G/G	9 (2.9)	6 (5.7)	1.99 (0.68–5.79)	0.23	2 (3.2)	1.24 (0.56–5.96)	1
rs16917237							
G/G	182 (59.1)	60 (56.6)	Reference		32 (50.8)	Reference	
G/T	105 (34.1)	39 (36.8)	1.13 (0.70–1.80)	0.63	27 (42.9)	1.46 (0.83–2.57)	0.24
T/T	21 (6.8)	7 (6.6)	1.01 (0.41–2.50)	1	4 (6.3)	1.08 (0.35–3.36)	1
rs11030119							
G/G	168 (55.6)	61 (57.5)	Reference		30 (47.6)	Reference	
G/A	122 (40.4)	36 (34)	0.81 (0.51–1.30)	0.41	29 (46)	1.33 (0.76–2.33)	0.39
A/A	12 (4)	9 (8.5)	2.06 (0.83–5.14)	0.13	4 (6.3)	1.87 (0.56–6.17)	0.48
rs10835210							
C/C	106 (34)	33 (31.1)	Reference		26 (41.3)	Reference	
A/C	137 (43.9)	55 (51.9)	1.29 (0.78–2.13)	0.38	28 (44.4)	0.83 (0.46–1.50)	0.65
A/A	69 (22.1)	18 (17)	0.60 (0.26–1.37)	0.63	9 (14.3)	0.53 (0.23–1.20)	0.18
rs6265							
Val/Val	195 (62.5)	62 (58.8)	Reference		34 (54)	Reference	
Val/Met	103 (33)	38 (35.9)	1.16 (0.73–1.85)	0.55	26 (41.3)	1.45 (0.82–2.54)	0.24
Met/Met	14 (4.5)	6 (5.7)	1.35 (0.50–3.66)	0.59	3 (4.8)	1.23 (0.33–4.50)	1
rs56164415 (-207C/T)							
C/C	281 (90.1)	96 (90.6)	Reference		52 (82.5)	Reference	
C/T	31 (9.9)	10 (9.4)	0.94 (0.45–2)	1	11 (17.5)	1.92 (0.91–4.05)	0.12

Odds ratio (OR) values with 95 % confidence intervals (CI) are shown

Single-SNP study

Genotyping analyses were successful in all the patients, whilst ten samples for SNP rs11030119, four for SNP rs16917237 and one for SNP rs11030102 failed to be genotyped in the control group. The frequencies of the six studied polymorphisms in the control group showed no departures from the Hardy–Weinberg equilibrium ($p > 0.05$ in all cases). The distribution of the different genotypes both in control subjects and ED patients is depicted in Table 3. None of the *BDNF* SNPs studied were associated with a greater risk of AN or BN (Table 3).

With regard to anthropometric parameters in the ED patients, analyses conducted using a recessive model of

Table 4 Effect of the rs16917237 SNP (G/T) on physiological parameters measured in patients with anorexia or bulimia nervosa

	AN ($n = 106$)		BN ($n = 63$)	
	GG + GT	TT	GG + GT	TT
N	99	7	59	4
Weight (kg)	44.25 ± 6.15	46 ± 6.41	57.93 ± 13.02	74.63 ± 16.58*
Height (m)	1.61 ± 0.07	1.59 ± 0.03	1.61 ± 0.06	1.61 ± 0.03
BMI (kg/m^2)	17.12 ± 1.92	18.34 ± 3.13	22.23 ± 4.77	28.94 ± 6.22*

Mean ± SD values are shown

* $p < 0.0083$ (significant value after correction for multiple testing considering the six SNPs assayed)

N number of subjects, *AN* anorexia nervosa, *BN* bulimia nervosa, *BMI* body mass index

inheritance revealed that the TT mutant homozygous genotype of the rs16917237 SNP was associated with higher BMI ($p < 0.01$) and higher minimum weight ($p < 0.01$) in the BN patients (Table 4). The statistical significance of the association remained after correction for multiple testing. No other SNP showed a relevant effect in any of the two study groups.

The study of the associations between *BDNF* genetic variants and personality characteristics and psychopathological symptoms in the AN group showed that patients carrying the -270CC wild type genotype scored significantly higher than CT carriers in the Interpersonal Distrust scale of the EDI-2 test (6.91 ± 4.89 vs. 2.67 ± 3.12, Table 5). In turn, BN patients who were carriers of the same genotype showed higher values in two of the three scales of the SCL-90R inventory, namely GSI (2.08 ± 0.81 vs. 1.44 ± 0.68, Table 6) and PST (73.31 ± 15.69 vs. 59.91 ± 20.0; Table 6). Finally, BN patients' carriers of the rs11030102 mutant homozygous genotype showed significantly higher scores in *social insecurity* than patients who harbored at least one wild type allele (7.83 ± 4.75 vs. 15.0 ± 1.41; Table 6). The *p* value for all these associations was significant after adjusting the results for multiple testing.

Haplotype study

Linkage disequilibrium (LD) plot and data (D, D' r and p) for the SNPs considered are depicted in supplementary Figure S1.

The haplotype analysis carried out by the sliding window approach using three contiguous SNPs produced four loci of interest, namely locus 1, with SNPs rs6265, rs11030102 and rs10835210; locus 2, which included rs11030102, rs10835210 and rs16917237; locus 3, with rs10835210, rs16917237 and C-270T and locus 4, containing the rs16917237/C-270T/rs11030119 combination.

As it was observed in the single-SNP study, there were no relevant differences in the distribution of these haplotypes between AN or BN patients and control subjects (data not shown). Only the haplotype GCG in locus 4 was found to be slightly more frequent in the control group (frequency = 0.527) than in BN patients (frequency = 0.428; Chi square $p = 0.044$). The complete analyses of the distribution of *BDNF* haplotypes in the study population are shown in supplementary tables S1 and S2.

The regression analyses of *BDNF* haplotypes in relation to psychopathological symptoms in ED patients revealed that in the AN group the allele combination CGC within locus 3 was associated with all three scales of the SCL-90R inventory, namely GSI (BETA = 0.34, $r^2 = 0.07$, $p < 0.01$), PST (BETA = 6.80, $r^2 = 0.04$, $p < 0.05$) and PSDI (BETA = 0.20, $r^2 = 0.04$, $p < 0.05$). Furthermore, this locus 3 (haplotype CGT) was also observed to be associated with the same three scales in the BN patients GSI (BETA = −0.65, $r^2 = 0.09$, $p = 0.01$), PST (BETA = −13.52, $r^2 = 0.09$, $p = 0.01$) and PSDI (BETA = −0.39, $r^2 = 0.06$, $p = 0.06$). After adjusting for multiple testing considering the four haplotypes involved (level of significance: $p < 0.0125$), only the association with the GSI scale in AN patients remained relevant. Notwithstanding, it should be noted that *p* values for both the GSI and PST scales in BN patients were on the border of statistical significance.

The scores of the EDI-2 inventory were largely unaffected by the genetic variability in the four loci considered (data not shown). Only the association previously observed for the *interpersonal distrust* subscale and the C-270T SNP in AN patients was maintained for two haplotypes that harbored this SNP, namely CGT in locus 3 (BETA = −4.559, $r^2 = 0.0516$, $p = 0.023$) and GTA in locus 4 (BETA = −4.292, $r^2 = 0.0503$, $p = 0.025$), although significance was lost after Bonferroni correction.

Discussion

Our results did not show any significant associations between any SNP in the *BDNF* gene and the risk of AN or BN. This finding is consistent with the currently only

Table 5 Association between the studied *BDNF* genotypes (recessive model) and psychopathological traits in anorexia nervosa patients measured by the EDI-2 and SCL-90R inventories

	rs56164415 (C-270T)		rs6225		rs11030102	
	C/C	C/T	V/V + V/M	M/M	C/C + C/G	G/G
EDI-2						
DT	11.19 ± 6.45	12.33 ± 5.94	11.28 ± 6.39	11.4 ± 6.88	11.46 ± 6.44	8.67 ± 5.13
B	3.02 ± 4.59	1.78 ± 3.23	2.94 ± 4.52	2.4 ± 4.34	3.02 ± 4.59	1.17 ± 1.6
BD	13.04 ± 8.5	11.67 ± 5.98	12.65 ± 8.29	18 ± 7.18	12.95 ± 8.3	12.5 ± 8.94
I	10.98 ± 8.02	6.56 ± 8.08	10.82 ± 8.17	6.0 ± 4.74	10.44 ± 8.01	12.83 ± 9.66
P	5.91 ± 3.95	6.11 ± 5.82	5.94 ± 4.09	5.8 ± 5.12	5.86 ± 4.1	7.0 ± 4.56
ID	**6.91 ± 4.89***	2.67 ± 3.12	6.61 ± 4.97	5.0 ± 3.16	6.46 ± 4.99	7.67 ± 3.08
IA	9.63 ± 7.2	6.11 ± 6.72	9.45 ± 7.3	6.6 ± 4.39	9.24 ± 7.21	10.33 ± 7.55
MF	8.46 ± 5.4	6.33 ± 4.8	8.28 ± 5.48	8.0 ± 2.35	8.22 ± 5.37	9.0 ± 5.73
A	6.56 ± 4.48	5.78 ± 4.12	6.34 ± 4.41	9.4 ± 4.16	6.55 ± 4.56	5.5 ± 1.05
IR	7.24 ± 5.88	5.78 ± 6.98	7.24 ± 6.05	4.6 ± 3.29	7.0 ± 6.0	8.83 ± 5.56
SI	7.6 ± 5.47	4.33 ± 4.06	7.39 ± 5.51	5.8 ± 3.56	7.27 ± 5.49	8 ± 4.69
Total	90.55 ± 45.22	69.44 ± 45.45	88.95 ± 46.16	83 ± 30.59	88.47 ± 46.39	91.5 ± 28.35
Bulimia						
GSI	1.6 ± 0.84	1.4 ± 0.88	1.6 ± 0.86	1.33 ± 0.47	1.57 ± 0.85	1.81 ± 0.62
PST	62.55 ± 21.25	62.11 ± 27.9	62.65 ± 22.16	59.8 ± 12.66	62.04 ± 22.04	69.83 ± 16.39
PSDI	2.2 ± 0.64	1.85 ± 0.61	2.18 ± 0.65	1.98 ± 0.48	2.16 ± 0.65	2.28 ± 0.45
	rs16917237		rs11030119		rs10835210	
	G/G +G/T	T/T	G/G + G/A	A/A	A/A + AC	C/C
EDI-2						
DT	11.33 ± 6.41	10.67 ± 6.41	10.79 ± 6.31	11.67 ± 6.47	10.93 ± 6.37	13.06 ± 6.33
B	2.97 ± 4.53	2.0 ± 4	2.37 ± 3.6	3.32 ± 5.05	2.55 ± 3.95	4.65 ± 6.38
BD	12.68 ± 8.33	16.67 ± 7.2	12.12 ± 8.31	13.53 ± 8.3	12.7 ± 8.3	14.0 ± 8.43
I	10.76 ± 8.19	7.83 ± 6.18	10.79 ± 8.29	10.42 ± 8.0	10.53 ± 7.87	10.82 ± 9.33
P	5.98 ± 4.09	5.17 ± 4.83	6.19 ± 4.55	5.74 ± 3.79	5.84 ± 4.22	6.35 ± 3.66
ID	6.53 ± 4.94	6.5 ± 4.64	6.51 ± 4.8	6.54 ± 5.01	6.58 ± 4.85	6.29 ± 5.29
IA	9.54 ± 7.29	5.67 ± 4.55	9.16 ± 7.04	9.42 ± 7.37	9.48 ± 7.28	8.47 ± 6.93
MF	8.29 ± 5.51	8.0 ± 2.1	8.05 ± 5.26	8.44 ± 5.49	8.06 ± 5.16	9.29 ± 6.35
A	6.37 ± 4.42	8.33 ± 4.55	5.63 ± 4.0	7.14 ± 4.66	6.17 ± 4.31	8.06 ± 4.81
IR	7.23 ± 6.08	5.17 ± 3.25	7.7 ± 6.48	6.67 ± 5.56	7.05 ± 5.81	7.41 ± 6.84
SI	7.38 ± 5.54	6.17 ± 3.31	7.67 ± 5.41	7.04 ± 5.47	7.49 ± 5.31	6.41 ± 6.07
Total	89.06 ± 46.39	82.17 ± 27.43	86.98 ± 43.24	89.91 ± 47.34	87.39 ± 43.7	94.82 ± 54.12
SCL90R						
GSI	1.6 ± 0.86	1.32 ± 0.42	1.68 ± 0.85	1.51 ± 0.84	1.59 ± 0.87	1.57 ± 0.7
PST	62.72 ± 22.27	59.17 ± 11.43	64.86 ± 22.54	60.74 ± 21.18	61.96 ± 22.54	65.18 ± 17.77
PSDI	2.18 ± 0.65	2.0 ± 0.43	2.19 ± 0.63	2.15 ± 0.65	2.18 ± 0.65	2.09 ± 0.59

Mean ± SD values are shown. Statistically significant associations are shown in bold

* $p < 0.0083$ (significant value after correction for multiple testing considering the six SNPs assayed)

DT drive for thinness, *B* bulimia, *BD* body dissatisfaction, *I* ineffectiveness, *P* perfectionism, *ID* interpersonal distrust, *IA* interoceptive awareness, *MF* maturity fears, *A* asceticism, *IR* impulse regulation, *SI* social insecurity, *GSI* global severity index, *PST* positive symptom total, *PSDI* positive symptom distress index

genome-wide association (GWAS) study performed in AN, which could not find any individual SNP associated with any definition of illness [34]. With regard to genetic association studies, most of the available information refers to either the Val66Met or the C-270T polymorphisms. The former has been associated with a higher risk for these

Table 6 Association between the studied *BDNF* genotypes (recessive model) and psychopathological traits in bulimina nervosa patients measured by the EDI-2 and SCL-90R inventories

	rs56164415 (C-270T)		rs6225		rs11030102	
	C/C	C/T	V/V + V/M	M/M	C/C + C/G	G/G
EDI-2						
DT	14.35 ± 4.82	16 ± 4.36	14.62 ± 4.82	15.5 ± 0.71	14.6 ± 4.81	16 ± 2.83
B	9.88 ± 7.23	11.09 ± 7.8	10.23 ± 7.31	6 ± 7.07	10.2 ± 7.3	7 ± 8.49
BD	19.14 ± 8.01	20.64 ± 7.45	19.37 ± 8.01	20.5 ± 0.71	19.45 ± 7.99	18 ± 4.24
I	12.27 ± 8.18	12.27 ± 7.55	12.43 ± 8.1	7.5 ± 0.71	11.98 ± 7.85	21 ± 11.31
P	6.55 ± 4.57	6.91 ± 4.06	6.57 ± 4.46	8 ± 5.66	6.63 ± 4.47	6 ± 5.66
ID	6.41 ± 4.64	6.55 ± 3.33	6.53 ± 4.45	3.5 ± 2.12	6.3 ± 4.26	10.5 ± 9.19
IA	14.1 ± 6.87	15.82 ± 5.49	14.53 ± 6.7	10.5 ± 2.12	14.22 ± 6.66	20 ± 1.41
MF	9.49 ± 6.03	7.27 ± 4.82	9.08 ± 5.88	9.5 ± 7.78	9.03 ± 5.91	11 ± 5.66
A	7.76 ± 4.41	6.91 ± 2.84	7.68 ± 4.17	5.5 ± 4.95	7.5 ± 4.19	11 ± 0.0
IR	9.98 ± 6.55	8.55 ± 6.06	9.83 ± 6.5	6.5 ± 3.54	9.7 ± 6.53	10.5 ± 3.54
SI	7.76 ± 4.82	9.45 ± 4.95	8.23 ± 4.84	3 ± 0	7.83 ± 4.75	**15.0 ± 1.41***
Total	117.71 ± 40.25	121.45 ± 35.92	119.12 ± 39.57	96 ± 25.46	117.45 ± 39.32	146 ± 35.36
SCL-90R						
GSI	**2.08 ± 0.81***	1.44 ± 0.68	1.96 ± 0.84	2.23 ± 0.46	1.95 ± 0.83	2.49 ± 0.33
PST	**73.31 ± 15.69***	59.91 ± 20	70.5 ± 17.29	80.33 ± 11.55	70.75 ± 17.28	77.5 ± 13.44
PSDI	2.48 ± 0.64	2.09 ± 0.47	2.41 ± 0.64	2.34 ± 0.12	2.39 ± 0.63	2.91 ± 0.12
	rs16917237		rs11030119		rs10835210	
	G/G + G/T	T/T	G/G + G/A	A/A	A/A + AC	C/C
EDI-2						
DT	14.6 ± 4.91	15.25 ± 0.5	15.88 ± 4.65	13.24 ± 4.53	14.93 ± 4.78	12.75 ± 4.3
B	10.09 ± 7.38	10.25 ± 6.7	10.15 ± 7.39	10.03 ± 7.29	10.13 ± 7.17	9.88 ± 8.51
BD	19.17 ± 8.07	22.75 ± 3.1	20.48 ± 6.67	18.17 ± 9.02	19.57 ± 8.16	18.25 ± 5.9
I	12.29 ± 8.16	12 ± 6.38	13.3 ± 8.56	11.1 ± 7.32	12.35 ± 8.11	11.75 ± 7.85
P	6.66 ± 4.5	6 ± 4.32	6.94 ± 4.78	6.24 ± 4.11	6.43 ± 4.42	7.88 ± 4.79
ID	6.48 ± 4.51	5.75 ± 2.99	6.09 ± 4.34	6.83 ± 4.54	6.11 ± 4.5	8.63 ± 3.25
IA	14.64 ± 6.79	11 ± 1.41	15.15 ± 6.41	13.55 ± 6.9	14.43 ± 6.78	14.25 ± 5.99
MF	9.22 ± 5.9	7.25 ± 5.74	8.27 ± 5.93	10.03 ± 5.75	8.8 ± 6.04	11.13 ± 4.29
A	7.76 ± 4.21	5.5 ± 3.11	7.27 ± 3.75	8 ± 4.63	7.44 ± 4.29	8.75 ± 3.2
IR	9.88 ± 6.6	7.5 ± 2.65	9.52 ± 5.98	9.97 ± 7.02	9.28 ± 6.48	12.75 ± 5.55
SI	8.28 ± 4.91	5 ± 2.45	8.42 ± 5.04	7.66 ± 4.67	7.91 ± 4.91	9.13 ± 4.58
Total	119.07 ± 40.21	108.25 ± 21.82	121.48 ± 38.52	114.83 ± 40.48	117.37 ± 40,63	125.13 ± 29.6
SCL90R						
GSI	1.96 ± 0.84	2.17 ± 0.51	1.9 ± 0.88	2.05 ± 0.76	1.94 ± 0.83	2.11 ± 0.84
PST	70.56 ± 17.29	77 ± 15.14	69.61 ± 18.57	72.47 ± 15.56	70.11 ± 17.88	76.11 ± 10.91
PSDI	2.41 ± 0.64	2.43 ± 0.18	2.35 ± 0.66	2.47 ± 0.59	2.4 ± 0.62	2.45 ± 0.67

Mean ± SD values are shown. Statistically significant associations are shown in bold

* $p < 0.0083$ (significant value after correction for multiple testing considering the six SNPs assayed)

DT drive for thinness, *B* bulimia, *BD* body dissatisfaction, *I* ineffectiveness, *P* perfectionism, *ID* interpersonal distrust, *IA* interoceptive awareness, *MF* maturity fears, *A* asceticism, *IR* impulse regulation, *SI* social insecurity, *GSI* global severity index, *PST* positive symptom total, *PSDI* positive symptom distress index

disorders, particularly restrictive AN (ANR) [9, 35–37]. However, we and others [10, 38–41] could not reproduce these results. Moreover, the analysis of a subset of ANR patients in our population (data not shown) did not reveal further significant associations. In the same manner, and consistent with previous studies [35, 40], we did not detect

any association between the risk of ED and the C-270T SNP. The present controversy on the role of *BDNF* genetic variants in the susceptibility to ED has recently been stressed by Hong et al., who suggest that phenotype assessment may play an important role [42]. Haplotype analyses in the *BDNF* gene could help explain these inconsistencies, but these studies are still scarce in the ED setting. Only a previous family-based association study has identified a 2-SNP haplotype associated with AN [43]; however, we could not confirm this finding in our sample.

We next tested the effect of *BDNF* genetic variability on the anthropometric parameters of the patients. The results showed that the TT mutant genotype of the rs16917237 SNP was consistently associated with higher weight and BMI in subjects with BN. There are no previously reported evidences linking this SNP to body weight in ED patients but, interestingly enough, the GIANT (Genetic Investigation of ANthropometric Traits) consortium (http://www.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium) has recently included this polymorphism in a list of potential markers of obesity. In this regard, it should be mentioned that BN patients tend to display higher BMI than controls and higher rates of premorbid obesity [44, 45].

The putative impact of *BDNF* genetic variants on body weight has recently been pointed out by a GWAS study in healthy subjects, which reported the association of the rs10767664 SNP with higher BMI [46]. Although we did not test the effect of this variant, rs10767664 is in strong LD ($r^2 > 0.75$) with the Val66Met SNP, whose effect was evaluated with negative results by both the present work and previous studies in ED patients [9, 47]. Larger studies in the ED setting are, therefore, needed to confirm the observation reported by the afore-mentioned GWAS study for the general population.

On the other hand, Ribases et al. have reported an increase in body weight for BN patients (with no previous AN) who were carriers of the -270T allele [9, 47], an association we did not observe in our study. This discrepancy could reflect phenotypic heterogeneity of our bulimic sample, which did include women with a history of AN, the course of which often includes the emergence of bulimic symptoms and a crossover to the full syndrome of BN [48]. In addition, interactions of *BDNF* SNPs with dopaminergic polymorphisms holding the potential to affect body weight could have also influenced our observations [25, 49]. Overall, the explanation of the modulation of body weight by *BDNF* variants is still unclear. A putative hypothesis has been given by Saito et al., who found a positive correlation between serum BDNF levels and BMI among females with BN [50]. Although the precise biochemical effect of the rs16917237 SNP, which we found to be associated with weight, is presently unknown, the fact that other SNPs in the intronic regions of *BDNF* can affect circulating levels of the protein in BN patients [22, 43] suggests that this could be an underlying mechanism for the observed up-regulation of weight and BMI.

The main objective of this work was to investigate whether *BDNF* genetic variability correlates with personality dimensions and/or psychopathological symptoms in ED patients. To our knowledge, this has only been addressed by one previous study in Polish subjects with AN, albeit only the Val66Met and C-270T SNPs were analyzed [41]. The authors concluded that the -270T allele was related to certain personality traits. In our group of AN patients, it was the -270C allele that correlated with *interpersonal distrust*, meaning reluctance to form close relationships. The different inventories utilized, ethnicity, or the different setting in which the studies were conducted (outpatients vs. inpatients) might be behind this controversy.

The sliding window approach applied in this work revealed a broad effect of one of the resulting four loci on the scores of the SCL-90R test. This inventory measures psychopathological symptoms that do not necessarily have to be intimately related to ED and are common to various psychiatric disorders. The observed association of certain *BDNF* haplotypes with these symptoms, the negligible effect on the traits measured by the EDI-2 questionnaire, specifically developed for ED patients, and the fact that these haplotypes were not relevant for the susceptibility to ED, suggest altogether that *BDNF* genetic variability may be important for psychopathological problems that could actually reflect comorbid disorders in ED patients.

Why genetic variations in the *BDNF* gene could lead to psychopathological symptoms in ED patients? Compelling evidence suggests that the pathophysiology of several mental illnesses could be the result of an alteration in the synaptic plasticity caused by an altered expression and release of BDNF [51–53]. Furthermore, Mercader et al. [43] have reported that certain *BDNF* haplotypes are able to modulate plasma protein concentrations in BN patients. Now, these concentrations have in turn been shown to correlate negatively with the scores of psychological inventories in ED patients [50, 54, 55]; therefore, it is tempting to speculate that altered BDNF levels resulting from the presence of certain haplotypes in our patients could have contributed to the development of the observed psychopathological traits. A fact that supports this hypothesis is that the same SCL-90R scales that correlated with BDNF plasma levels in the study by Mercader et al. [55] were those affected by the locus identified in our study.

There are a number of limitations in the present work that should be considered. First, the limited size of our sample, particularly of the BN group, which may limit the

generalizability of the results. On the other hand, this limited sample also allowed for all the patients to be diagnosed and treated by the same clinicians in the same facilities over a short period of time, which increases the homogeneity of our analyses. Second, we did not consider ED clinical categories to correct for multiple testing, as this has been suggested to be too stringent to detect a moderate correlation with different endophenotypes in similar studies [55]. Third, our control sample was composed by University students, which might constitute a potential selection bias in terms of differences in socioeconomic status between patients and controls.

In conclusion, the findings of the present study, preliminary as they are given the described limitations, indicate that variability in the *BDNF* gene locus may contribute to anthropometric features, but also to psychopathological symptoms in ED patients that are likely indicative of comorbid disorders. Further studies with larger and homogeneous populations of patients, evaluated with the same inventories, are needed to confirm the reported associations.

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Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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ORIGINAL ARTICLE

Impact of *NEGR1* genetic variability on psychological traits of patients with eating disorders

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Genetics variants in the *NEGR1* gene, strongly expressed in the brain, have been reported to affect the neuronal control of food intake therefore inducing obesity. With the same rationale, we hypothesized that this genetic variability may be associated with psychological traits commonly displayed by eating disorder (ED) patients and/or with the risk for the disorder. We analyzed 21 tag-single-nucleotide polymorphisms (SNPs) in the coding sequence and adjacent regions of the *NEGR1* gene. A total of 169 ED patients (106 with anorexia nervosa (AN) and 63 with bulimia nervosa (BN)) and 312 healthy subjects were genotyped. Personality traits and general psychopathological symptoms were assessed by the Eating Disorders Inventory Test-2 (EDI-2) and Symptom Checklist 90 Revised inventories. None of the SNPs or haplotypes analyzed were associated with a greater risk of ED or correlated with anthropometric parameters. However, in patients with BN, four SNPs (rs12740031, rs10789322, rs6659202 and rs591540) correlated with the scores in Drive for Thinness (DT), Ineffectiveness (I) and Interoceptive Awareness (IA) (Bonferroni-$P < 0.05$ in all instances). The first two SNPs along with rs954299 and rs2422021 formed a haplotype block, which showed a consistent association with the EDI-2 score in BN patients (Bonferroni-$P = 0.01$). A subsequent three-SNP sliding-window approach identified a central area, encompassing both the haplotype block and the individually relevant SNPs that strongly correlated with the scores of BN patients in DT, I, IA and Bulimia. No associations were identified in the AN group. These preliminary results indicate that *NEGR1* could be an important locus influencing certain personality dimensions in BN patients.

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INTRODUCTION

Anorexia nervosa (AN) and bulimia nervosa (BN) are complex psychiatric disorders with an important genetic influence and disturbances of neurotransmitter, neuropeptide and neuroendocrine systems.[1] A number of central genes present in the central nervous system and involved in the regulation of eating behavior and body weight have been pointed out as good, however untested, candidates in studies with eating disorder (ED) patients.[2,3] One of such genes is the neuronal growth regulator 1 (*NEGR1*), which codes for a cell adhesion molecule of the immunoglobulin superfamily that belongs to the IgLON subgroup. *NEGR1* is strongly expressed in various brain regions including the cerebral cortex and hypothalamus,[4] where it seems to be involved in neurite outgrowth.[5] The specific function of the gene is presently unknown, although experiments in mice with abolished *NEGR1* function indicate a possible role in the neuronal control of body weight and food intake.[4]

In this regard, several human genome-wide association studies indicate that the *NEGR1* gene may be an important obesity locus.[6–10] Subsequent studies have replicated this observation in different cohorts.[11–13] On the other hand, there also are indications that *NEGR1* could also be involved in psychiatric disorders. For instance, Maccarrone *et al.*[14] have recently identified differences in the cerebrospinal fluid levels of the NEGR1 protein between healthy subjects and depressed and bipolar patients.

It has been reported that genetic variability in brain-expressed genes associated with obesity such as *FTO* or *MC4R* can also be related to ED.[15,16] Accordingly, we hypothesize that polymorphisms in or close to the *NEGR1* gene locus could be involved in ED-related behaviors through similar neuronal mechanisms.

In order to test this hypothesis, we have analyzed 21 tag-single-nucleotide polymorphisms (SNPs) in the coding sequence and adjacent 3′- and 5′-untranslated regions of the *NEGR1* gene in a population of ED patients and healthy subjects and identified associations with the susceptibility for the disorder, anthropometric parameters and psychopathological traits.

MATERIALS AND METHODS

Subjects

The study group consisted of 169 unrelated consecutive female patients with AN ($n = 106$) or BN ($n = 63$), some of whom had also participated in previous studies by our group.[17–19] The patients attended the collaborating Eating Disorders Unit at the Mental Health Outpatient Clinic in Badajoz (Spain), and were diagnosed by one psychiatrist and one psychologist using the ED section of the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, 4th edn.[20] Anthropometric (weight, height and body mass index) and psychological parameters (see below) were collected. Diagnosis was blind to genotype. Exclusion criteria, determined upon screening, included dementia, mental retardation, schizophrenia, Turner's syndrome, other neurological disorders and underlying endocrine pathologies.

A total of 312 healthy women from the same geographical area as the patients (Health District of Badajoz, Spain) were recruited among University students and staff. Interviews were carried out to guarantee that they had never been diagnosed as having any psychiatric disorder or received any

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psychiatric treatment. None of the participating control subjects showed anthropometric parameters indicative of present ED.

All the participants were white Spanish individuals who gave written informed consent. The study protocol was approved by the Ethics Committee of the University of Extremadura and was conducted in accordance with the Declaration of Helsinki and its subsequent revisions.

SNPs selection

European population (CEU) SNP data were downloaded from the International Haplotype Mapping Project website (http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap27_B36). We analyzed the coding sequence and adjacent 3′- and 5′-untranslated regions of the *NEGR1* gene (ref. NC_000001.10) and tag SNPs were assigned by using Haploview v4.2 software (Daly Lab at the Broad Institute, Cambridge, MA, USA) (Table 1). The minor allele frequency considered was 10%, and pair-wise tagging with a minimum r^2 of 0.80 was applied to capture common variations. A set of 24 tag SNPs was thus selected of which three (rs2630391, rs6696052 and rs11209817) did not survive quality-control with the genotyping methods. All the SNPs were intronic and located within an 824.4-kb region (chromosome positions 71910991 to 72735458) encompassing the *NEGR1* gene

Genotype analysis

Blood samples from all participants were collected and stored at − 80 °C until analysis. Genomic DNA was isolated from peripheral blood leukocytes in 2-ml aliquots of whole-blood samples with a Qiagen blood midi kit (Qiagen, Chatsworth, CA, USA). The purified DNA samples were then stored at 4 °C in sterile plastic vials.

Genotype analyses for SNPs determination were performed with the single-base extension polymerase chain reaction Sequenom iPLEX-Gold and the mass spectrometry-based platform MassARRAY MALDI-TOF (Sequenom, San Diego, CA, USA) at the Spanish Genotyping National Centre (CEGEN-ISCIII). In brief, the analyses consisted of an initial locus-specific PCR, followed by single-base extension using mass-modified dideoxynucleotide terminators of an oligonucleotide primer, which anneals immediately upstream of the polymorphic site of interest. Using matrix-assisted laser desorption ionization time-of-flight mass spectrometry, the distinct mass of the extended primer identifies the allele.[21]

Study of psychological traits

ED patients were evaluated on the first visit to the collaborating Eating Disorders Unit by experienced clinicians. The patients completed the Eating Disorders Inventory Test-2 (EDI-2) and the Symptom Checklist 90 Revised self-reported questionnaires. EDI-2 was designed to assess ED-related cognitive and behavioral characteristics by initially measuring eight main subscales (Drive for Thinness (DT), Bulimia, Body Dissatisfaction, Ineffectiveness, Perfectionism, Interpersonal Distrust, Interoceptive Awareness (IA) and Maturity Fears), to which other three (Asceticism, Impulse Regulation and Social Insecurity) were added in a second version of the inventory.[22] The EDI-2 test has been validated in the Spanish population showing high internal consistency between the different subscales.[23] The Symptom Checklist 90 Revised inventory, which has also been validated in Spaniards,[24] assesses a broad range of psychopathological symptoms. Three indexes (Global Severity Index; Positive Symptom Distress Index; and Positive Symptom Total) and scores in nine primary symptom dimensions (somatization, depression, anxiety, hostility, phobic anxiety, paranoid ideation and psychoticism) are obtained upon completion of the questionnaire.[25]

Table 1. Single-nucleotide polymorphisms genotyped in the region encompassing the *NEGR1* gene

SNP	*Position*	*Alelles*	*MAF*
rs1983121	71910991	A/T	0.209
rs7553624	71961690	G/T	0.209
rs357202	71978773	A/C	0.284
rs1041639	72016278	C/T	0.191
rs1413368	72058552	A/G	0.005
rs928615	72077549	A/C	0.312
rs954299	72121585	G/T	0.456
rs2422021	72130161	C/T	0.441
rs10789322	72178928	A/G	0.298
rs12740031	72222487	A/G	0.301
rs6659202	72307420	A/T	0.244
rs591540	72331815	A/C	0.459
rs3851882	72345350	C/T	0.203
rs2114214	72388052	A/G	0.428
rs2186096	72440878	C/T	0.24
rs12409966	72539545	C/T	0.486
rs12091740	72553991	C/T	0.476
rs7517923	72614077	G/T	0.474
rs10493494	72670904	C/G	0.246
rs1026566	72709042	C/T	0.449
rs12137231	72735458	C/T	0.463

Abbreviations: MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

Statistical analyses

Single-marker association analyses were carried out using logistic regression models adjusted for age using the *SNPassoc* R package.[26] The statistical power of the sample size was evaluated with a log-additive genetic model, analyzing the frequency for carriers of the variant alleles with an arbitrarily established effect size set at 2.0 (type I error = 0.05). With the available sample size, the statistical power for detecting associations with categorical variables ranged from 0.80 to 0.87 in BN and 0.93 to 0.97 in AN, depending on the minor allele frequency (Quanto software v. 1.2.4, University of Southern California). The study was, however, underpowered in the case of rs1413368, which displayed a very low frequency of 0.005.

Haplotype blocks were identified with Haploview v4.2 and associations with disease were assessed using PLINK v1.07 (ref. 27) with a haplotype frequency cutoff of 0.1. To refine the haplotype construction and to identify core regions with the potential to affect either the susceptibility for ED or the anthropometric/psychopathological parameters in the patients, we used a sliding-window approach to construct successive and adjacent three-SNP haplotypes. The *P*-value for the statistical significance of all observed associations was set at 0.0023 after Bonferroni correction for multiple testing.

RESULTS

Clinical and descriptive characteristics of ED patients and healthy subjects are summarized in Table 2. Control subjects displayed significantly higher weight and body mass index than AN patients ($P < 0.05$). In addition, BN patients scored significantly higher than AN patients in the different scales measured in the psychometric evaluation (Table 2).

Single-marker analyses

Minor allelic frequencies for the 21 polymorphisms assayed in the study population ranged from 0.005 to 0.486 (Table 1). None of the SNPs analyzed were individually associated with a greater risk of ED or altered anthropometric parameters after Bonferroni correction of the data (data not shown).

However, we observed a consistent association between four *NEGR1* genetic variants and the scores of the EDI-2 test in the psychometric evaluation of the BN patients. First, two SNPs that were in complete linkage disequilibrium in this group, rs10789322 and rs12740031, were associated with the scores of the IA scale, with statistically significant differences between wild-type, heterozygous and mutant homozygous subjects (Table 3). In the same manner, the rs6659202 and rs591540 SNPs were associated with Ineffectiveness and DT, respectively (Table 3). Three other SNPs (rs1983121, rs12137231 and rs3851882) were related to at least one of the scales, but statistical significance was lost after correction for multiple testing (data not shown).

Haplotype study

The linkage disequilibrium plot for *NEGR1* in the whole population of the study is depicted in Supplementary Figure 1. Mirroring the

Table 2. Descriptive and clinical variables of patients with AN or BN and healthy controls

	Controls	*ED*	*AN*	*BN*	P-*value*
Anthropometric parameters					
Weight (kg)	58.3 ± 10.12	49.73 ± 11.94	44.28 ± 6.08	59.16 ± 13.65	< 0.05[a]
BMI (kg m^{-2})	21.99 ± 4.25	19.21 ± 4.34	17.19 ± 2.01	22.71 ± 5.03	< 0.05[a]
Height (m)	1.62 ± 0.07	1.61 ± 0.06	1.60 ± 0.07	1.61 ± 0.06	NS
Age (years)	21.90 ± 7.01	20.1 ± 7.9	18.9 ± 6.2	20.9 ± 8.1	NS
Psychometric evaluation					
Total EDI-2 score			88.84 ± 45.53	117.27 ± 39.14	< 0.0001[b]
GSI (SCL-90R) score			1.62 ± 0.85	1.96 ± 0.82	< 0.05[b]
PST (SCL-90R) score			63.33 ± 21.91	71.11 ± 17.07	< 0.05[b]
PSDI (SCL-90R) score			2.18 ± 0.64	2.4 ± 0.62	< 0.05[b]

Abbreviations: AN, anorexia nervosa; BN, bulimia nervosa; BMI, body mass index; ED, eating disorder; EDI-2, Eating Disorders Inventory Test-2; GSI, Global Severity Index; PSDI, Positive Symptom Distress Index; PST, Positive Symptom Total; SCL-90R, Symptom Checklist 90 Revised. Mean ± s.d. values are shown. [a]*P*-value for the X^2 comparison between controls and AN patients. *bP*-value for the comparison between BN and AN patients (*T*-test).

Table 3. Single-SNP approach showing polymorphisms with a relevant effect on the psychometric evaluation carried out in the bulimia nervosa patients (codominant model)

	rs10789322/rs12740031[a]			*rs6659202*			*rs591540*		
Scale	*A/A*	*A/G*	*G/G*	*A/A*	*A/T*	*T/T*	*A/A*	*A/C*	*C/C*
DT	15.25 ± 2.50	12.95 ± 1.04	15.61 ± 0.73	10.0 ± 0.0	13.83 ± 0.90	15.53 ± 0.80	[b]16.83 ± 0.55	15.42 ± 0.72	10.72 ± 14.76
I	5.75 ± 2.1	9.27 ± 1.77	14.83 ± 1.20	[b]15.78 ± 1.4	8.69 ± 1.20	4.0 ± 0.0	12.5 ± 1.37	12.97 ± 1.43	10.29 ± 2.37
IA	[b]17.25 ± 0.92	11.32 ± 13.40	5.75 ± 0.95	3.0 ± 0.0	12.69 ± 1.16	16.31 ± 1.13	16 ± 1.12	14.75 ± 1.13	12.14 ± 2.06

Abbreviations: BN, bulimia nervosa; DT, Drive for Thinness; I, Ineffectiveness; IA, Interoceptive Awareness; SNP, single-nucleotide polymorphism. Mean ± s.e. values are shown. [a]rs10789322 (A/G) and rs12740031 (A/G) were in complete linkage disequilibrium in the BN patients and hence they showed the same values for the measured scales. [b]$P < 0.05$ for the difference between the three genotypes after Bonferroni correction for multiple testing.

results from the single-SNP study, there were no relevant differences in the distribution of the different haplotypes between AN or BN patients and control subjects (data not shown).

Interestingly, the results of the psychometric evaluation in the BN group were again subjected to influence by *NEGR1* haplotypes. The haplotype analysis summarized in Figure 1 revealed two relevant regions, a two-SNP block encompassing 17 kb and a larger block encompassing 100 kb and including four SNPs (rs954299, rs2422021, rs10789322 and rs12740031). Haplotype GCAA in this second block displayed a strong association with higher total scores in the EDI-2 inventory (Figure 1). In order to investigate which specific EDI-2 scale(s) was/were behind this observation, we applied a sliding-window approach and analyzed the associations of the resulting 19 loci of interest with the scores obtained in the questionnaires. Figure 2 shows *P*-values for the correlation of these three-SNP combinations with several personality dimensions measured by the EDI-2 test. A central area spanning 210 kb can be easily discerned that was associated with the scores of DT, Bulimia, IA and Ineffectiveness. It is noteworthy that this central area encompassed the four SNPs included in the formerly described haplotype block 2 (Figure 2). Only one more marker combination, upstream of the *NEGR1*-coding region (rs12091740–rs7517923–rs10493494), was associated with the results of IA (Figure 2).

DISCUSSION

Genetic alterations in the complex neural system aimed to keep energy balance by food intake are known to trigger and maintain obesity. However, it has also been hypothesized that the genes involved in the neuronal control of weight regulation may also be relevant for ED.[17,28–30] In this regard, the putative impact of the *NEGR1* genetic variants, whose loci have repeatedly been shown to be associated with the risk for obesity[6–13] remained to be tested in the field of ED.

The main finding of this study was that genetic variability in a region within the *NEGR1* gene locus showed a profound impact in the scores obtained by BN patients in various personality dimensions. The mechanisms underlying the different behaviors displayed by patients with psychiatric disorders are extremely complex, but it is known that some of these mechanisms may be mediated by the contribution of genes to psychological traits.[31,32] In this regard, the trait most profoundly affected by *NEGR1* genetic variability in the BN patients was IA, which measures the ability of an individual to discriminate between sensations and feelings, and, most interestingly, between the sensations of hunger and satiety.[22] Interoceptive deficits have been suggested to be one factor that bridges the gap between abnormal functioning in interoceptive neural networks and symptom presentation in BN, as binging and purging may reflect a difficulty in internally regulating misinterpreted hunger and satiety cues.[33] Indeed, several studies have reported that those with BN struggle to detect various body cues.[34–36] This hypothesis is supported by imaging studies that identified irregular patterns of activation in the brain, when women with BN are given interoceptive taste stimuli.[37] Being *NEGR1* a gene expressed predominantly in brain with an important, although semi-unknown, role in neuronal growth, we propose that genetic variability in its locus might contribute to this altered interoceptive sensitivity.

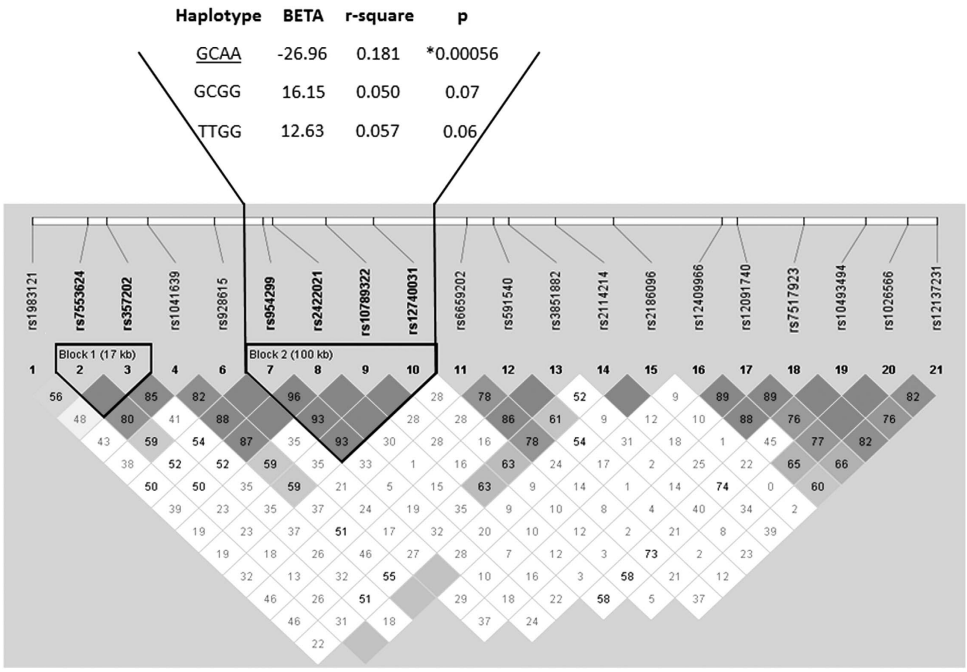

Figure 1. Linkage disequilibrium (D') pattern among 21 selected single-nucleotide polymorphisms (SNPs) in bulimia nervosa patients. SNPs are shown by location as in Table 1. Squares without numbers represent D' values of 1.0; all numbers represent the D' value expressed as a percentile. Dark squares represent pairs with LOD score for linkage disequilibrium of $\geqslant 2$, light-gray squares represent D' = 1 but LOD < 2 and white squares represent LOD < 2 and D' < 1.0. Different allele combinations for block 2 are also shown, as well as β, r^2 and *P*-values for the association with the scores of the Eating Disorders Inventory-2. *Association remained significant after correcting for multiple testing.

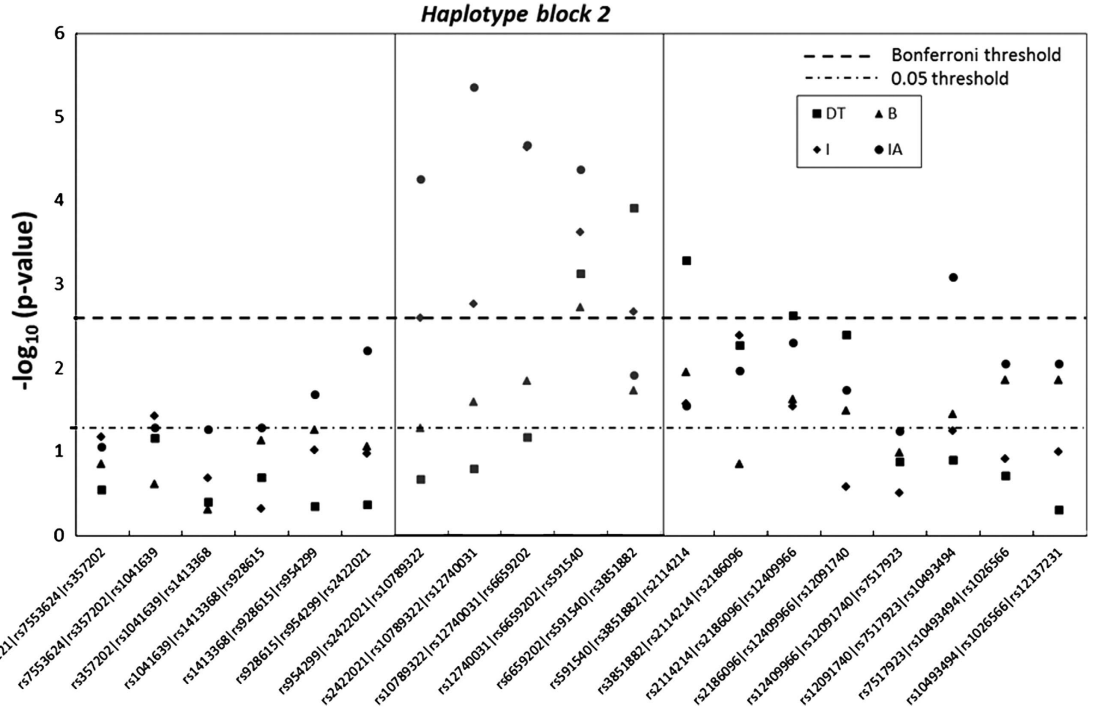

Figure 2. Sliding-window approach combining three consecutive SNPs for the association with personality dimensions in bulimia nervosa patients. The shaded area corresponds to the previously identified haplotype block 2 encompassing a 100-kb area with single-nucleotide polymorphisms rs954299 to rs3851882. B, bulimia; DT, drive for thinness; I, ineffectiveness; IA, interoceptive awareness.

It is also noteworthy that the interoceptive deficit seems to be higher in BN than in AN or obese patients.[38] This is consistent with the lack of association observed between *NEGR1* variants and this trait in our AN group, and with the fact that BN patients in our series displayed far worse IA than did women with AN (EDI-2 scores: 14.18 ± 6.64 vs 9.38 ± 7.23; $P < 0.0001$).

Interestingly, Klabunde *et al.*[33] found that the interoceptive-processing deficit is also present in women that recovered from

BN, and the authors rightfully raised the question whether this deficit could be a consequence of having suffered from BN or a biological trait that is present prior to the development of bulimic symptoms. Our results describing a genetic alteration as a putative source of failure to correctly process hunger/satiety sensations would support their latter theory. Moreover, the connection between genetic variability in brain-expressed genes, eating disorders and IA is not unprecedented in the literature. We and others have suggested that *BDNF*, a gene also involved in neuronal growth and stability, may influence the severity of symptoms in ED by modulating the associated psychopathology,[17,39] in particular through the impairment of IA.[39]

Ineffectiveness, which assesses feelings of inadequacy, insecurity, worthlessness and having no control over one's life, was the other EDI-2 scale most deeply affected by *NEGR1* genetic variability in the BN patients. The concept of Ineffectiveness, or poor self-esteem, as a risk factor for ED is still controversial,[40,41] but it has been suggested that this trait could actually be the mediator of other risk factors in BN such as childhood abuse,[42,43] an information that unfortunately was not available in our study. Goethals *et al*.[44] have demonstrated that pathological scores in Ineffectiveness are supported by biological processes in the brain, as the scores correlate with cerebral blood flow in brain regions with a known role in foregoing functions, such as cortical areas. Given the lack of knowledge on the specific function of *NEGR1* and the mechanisms by which this gene can affect behavior, it is difficult to establish a hypothesis for a link between variability in its locus and Ineffectiveness. Interestingly enough, however, this trait and DT, which was also affected by *NEGR1* variability in our sample, have been reported to correlate with genetic variants of other brain genes.[45–47] Moreover, and in line with our results, it has been shown that the connections among genotypes and these character scales were more expressed in BN patients than in women with AN.[47]

On the other hand, the results showed no significant increased risk for BN (or AN) associated with any SNP or haplotype, which suggests that the affected traits would be more important in terms of severity or duration of the disorder than in terms of increased susceptibility, where environmental and social factors must have an important role. In the same manner, we did not observe an effect of *NEGR1* variants on either the weight or body mass index of our BN group. It would be very difficult to demonstrate an association of these SNPs with a tendency to obesity in bulimic patients, as their impact would likely be counteracted by compensatory mechanisms, for example, purging or extreme physical activity.

Several limitations have to be considered in this study. First, the relatively small size of the population studied, particularly of the BN group, might affect the generalizability of these results and therefore the findings presented herein should be considered as preliminary. On the other hand, this limited sample also allowed for all the patients to be diagnosed and treated by the same clinicians in the same facilities over a short period of time, which reduced the chance that the findings may be due to population structure. Second, we could not determine three of the tag SNPs initially established and therefore the relevance of the regions tagged by these variants could not be assessed. Finally, we did not consider the different psychopathological scales to correct for multiple testing, as we did with the 21 SNPs assayed, as this procedure has been suggested to be too stringent to detect a moderate correlation with different endophenotypes in similar studies.[48]

It should also be mentioned that, although the *NEGR1* gene locus has been repeatedly associated with changes leading to obesity, which constitutes the backbone of our hypothesis, some studies have failed to reproduce this association.[49,50] In the same manner, it must not be ruled out that the *NEGR1* variants were in fact reflecting long-range associations with other genes. It has recently been reported that the increased risk of obesity conferred by a deletion in the *NEGR1* gene was in fact driven by a neighboring 8-kb deletion (rs1993709)[10] comprising the conserved transcription factor-binding site for NKX6.1, which is also involved in neuronal development.[51]

However, even acknowledging these limitations, there are some facts that highlight the significance of the findings described herein. First, both the single-SNP study and the two different multiple-SNP approaches pointed to the same region as an influential locus for ED-related psychological traits. Second, the *P*-values obtained for these associations survived multiple testing correction for 21 SNPs, and third, there is recent evidence relating NEGR1 protein levels to depression and other psychiatric disorders that could also be present in ED patients.[14]

These results taken together, preliminary as they are given the described limitations, indicate that the *NEGR1* gene could be an important locus influencing certain personality dimensions in BN patients, particularly IA, DT and Ineffectiveness. Further studies with larger and homogeneous populations of patients evaluated with the same inventories are nevertheless warranted to confirm the reported associations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Melanocortin-4 receptor gene variants are not associated with binge-eating behavior in nonobese patients with eating disorders

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We aimed to determine whether variability in the melanocortin-4 receptor (*MC4R*) gene, predisposing to hyperphagia and obesity, may also be present in nonobese patients with binge-eating behavior or be related to anthropometric or psychopathological parameters in these patients. The coding region of the *MC4R* gene was sequenced in nonobese patients with binge-eating behavior diagnosed with bulimia nervosa or binge-eating disorder ($n = 77$); individuals with severe early-onset obesity ($n = 170$); and lean women with anorexia nervosa ($n = 20$). A psychometric evaluation (Eating Disorders Inventory-2 and Symptom Checklist 90 Revised inventories) was carried out for all the patients with eating disorders. In the obesity group, 10 different variants were identified, whereas in the binge-eating patients, only two individuals with bulimia nervosa were found to carry the I251L polymorphism, which did not correlate with weight, BMI, or psychopathological features. We found no evidence that mutations in the *MC4R* gene are associated with binge-eating behavior in nonobese eating disorder patients. *Psychiatr Genet* 25:35–38

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Keywords: binge-eating, bulimia, *MC4R*, obesity, polymorphisms

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Introduction

The melanocortin-4 receptor (MC4R) is a seven-transmembrane domain receptor expressed in the hypothalamus that activates adenylate cyclase in response to the α-melanocyte-stimulating hormone, thus mediating satiety signals (Mountjoy *et al.*, 1994). Early animal studies showed that inactivation of the *MC4R* gene resulted in obesity associated with hyperphagia (Huszar *et al.*, 1997). Since then, this gene has been analyzed intensively in molecular genetic obesity research. Indeed, *MC4R* mutations constitute the most common monogenic cause of human obesity, with up to 4% of morbid obesity cases attributable to mutations in this gene (Vaisse *et al.*, 2000; Farooqi and O'Rahilly, 2006). To date, more than 150 different *MC4R* mutations, mostly leading to a reduced function, have been detected in obese individuals (Hinney *et al.*, 2013).

Obesity is also perceived as a risk factor for the development of bulimia nervosa (BN) (Fairburn *et al.*, 1997). Therefore, it is reasonable to believe that genetic alterations predisposing to obesity can be more frequently found in BN patients than in healthy individuals. In this respect, Hebebrand *et al.* (2002) reported the first case of a mutation in the *MC4R* gene predisposing to obesity in a BN patient. In addition, the same group reported the first observation of binge-eating disorder (BED) associated with *MC4R* genetic alterations (Sina *et al.*, 1999). However, since these initial works, several studies have reported contradictory results on the association between binge-eating behavior and *MC4R* mutations in eating disorder (ED) patients (Branson *et al.*, 2003; Hebebrand *et al.*, 2004; Lubrano-Berthelier *et al.*, 2006; Valette *et al.*, 2014).

Although according to the recently implemented *Diagnostic and Statistical Manual of Mental Disorders* (DSM)-V, BED and BN are different clinical entities, binge-eating symptoms are present in both (Call *et al.*, 2013). In the present work, we have screened the coding region of the *MC4R* gene to detect genetic differences between nonobese patients with binge-eating behavior who had been diagnosed with BN or BED, obese individuals, and, for comparison purposes, lean women diagnosed with anorexia nervosa (AN). In addition, we carried out a psychometric evaluation of the ED patients to determine whether the *MC4R* mutations could be related to specific psychopathological features.

Participants and methods

Participants

First, 170 unrelated patients with severe early-onset obesity, whose pathogenesis could be attributed primarily to

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genetic alterations, were screened for variations in the *MC4R* gene as a part of family studies on morbid obesity in Extremadura (Spain) (Gonzalez *et al.*, 2014; Albuquerque *et al.*, 2014). Genetic contribution was suspected on the basis that (i) all these participants showed a weight greater than three SD before 14 years of age, (ii) maintained their obesity throughout their lifetime, and (iii) referred at least two other morbid obesity cases among first-degree or second-degree relatives. Individuals with signs and/or symptoms of syndromic obesity were excluded. Second, a small group of 20 lean women with AN was also included to investigate putative genetic differences between patients with 'extreme' weights, as described previously (Gotoda *et al.*, 1997). Finally, the third study group included 77 unrelated consecutive nonobese patients with binge-eating behavior diagnosed with either BN or BED. All the ED patients were diagnosed by one psychiatrist and one psychologist using the ED section of the Structured Clinical Interview for DSM-IV (First *et al.*, 1996), but diagnoses were later re-evaluated to comply with the new DSM-V guidelines. Diagnosis was always blind to genotype. The examination of endophenotypes in ED patients was performed using two self-reported questionnaires, namely, the Eating Disorders Inventory-2 (EDI-2) and the Symptom Checklist 90 Revised (SCL-90R). ED patients were White Spanish women recruited in the same geographical area as the obese patients (Extremadura, Southwest Spain).

All procedures followed were in accordance with the ethical standards of the Bioethics Committee of the University of Extremadura and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for inclusion in the study.

Sequence analysis

Genomic DNA was extracted from peripheral blood using the QIAamp DNA Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Bidirectional sequence analysis of genomic DNA was carried out encompassing the entire single coding exon sequence of the *MC4R* gene (chromosome 18: 58 038 564–58 040 001; ENSG00000166603). Three primer pairs [primer3 software (Koressaar and Remm, 2007)] were utilized: MC-1, 5′-TGAGACGACTCCCTGACCCAG-3′ (forward)/5′-CACTGTGAAACTCTGTGCATC-3′ (reverse); MC-2, 5′-GATCACCCTATTAAACAGTACAG-3′ (forward)-/5′-TTGGCGGATGGCACCAGTGC-3′ (reverse); and MC-3, 5′-AGGCTTCACATTAAGAGGATTG-3′ (forward)/5′-TACAATATTCAGGTAGGGTAAGA-3′ (reverse). The PCR amplification was carried out in 25 µl of a total volume containing 12.5 µl BioMix (Bioline, London, UK), 0.4 µmol/l each primer, and 150 ng/µl template. The PCR conditions for the three primer sets included an initial denature step for 5 min at 94°C, followed by 35 cycles consisting of 45 s at 94°C, 45 s at 62°C, and 45 s at 72°C, with a final extension for 10 min at 72°C.

All amplified fragments were purified with ExoSAP-IT (GE Healthcare, Piscatway, New Jersey, USA) and both forward and reverse strands were subsequently subjected to Sanger's dideoxy chain termination sequencing reaction using the Big-Dye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, USA) and processed using an ABI 3130 automatic sequencer (Applied Biosystems). Base calling was performed with Sequencing Analysis v.5.2 (Applied Biosystems).

Statistical analyses

The comparison of allele frequencies between study groups was performed using the χ^2-test. Differences in anthropometric or psychopathological parameters between carriers and noncarriers were assessed using the *T*-test after confirming that the data showed a normal distribution (SPSS 15.0; SPSS Inc., Chicago, Illinois, USA). Data are presented as mean ± SD unless stated otherwise. In all instances, differences were considered to be significant when *P* values were less than 0.05.

Results and discussion

The anthropometric and psychopathological parameters of the participants are summarized in Table 1. In the obese patients, 10 different missense variants were identified in 12 different patients (Table 2). The overall frequency was 3.8%, which was similar to those reported previously for individuals with early-onset obesity (Farooqi *et al.*, 2003; Lubrano-Berthelier *et al.*, 2006). All carriers were heterozygous for a single nucleotide change, except one compound heterozygous individual, who carried both the S127L and the G252S polymorphism. In contrast, only two BN patients of the 77 nonobese women with binge-eating behavior were found to carry an *MC4R* variant, being heterozygous for the same polymorphism I251L (Table 2). The overall allele frequency in this group was significantly lower than that of the obesity group (1.3 vs. 3.8%; $P < 0.05$) (Table 2). However, the confidence intervals of the odds ratio for the overall effect size were far too wide to obtain statistical significance [odds ratio 3.34 (0.36–30.71)]. No mutations were detected in the lean women with AN.

Table 1 Characteristics of the sampled patients screened for mutations

	Obese	Binge-eating	AN
Individuals	170	77	20
Age (years at inclusion)	16.01 ± 13.20	17.71 ± 4.81	15.50 ± 2.30
Female (%)	61.2	100	100
Height (m)	1.57 ± 0.14	1.61 ± 0.05	1.60 ± 0.07
Weight (kg)	93.81 ± 28.37	61.33 ± 17.91	42.16 ± 6.18
BMI	37.13 ± 6.64	23.40 ± 6.20	16.40 ± 1.59
EDI-2 total score	–	117.75 ± 38.53	86.72 ± 36.40
SCL-90R GSI	–	1.96 ± 0.81	1.37 ± 0.73

AN, anorexia nervosa; EDI-2, Eating Disorders Inventory-2; GSI, Global Severity Index; SCL-90R, Symptom Checklist 90 Revised.

Table 2 ***MC4R*** **mutations found in the binge-eating (*n* = 77) and obese (*n* = 170) patients**

Nucleotide changes	rs number	HGMD reference	AA change	Obese [*N* (%)]	Binge-eating [*N* (%)]
c.20G > A	rs142837166	CM035774	R7H	1 (0.29)	–
c.95G > A	NA	CM070989	G32E	1 (0.29)	–
c.227A > G	rs199558727	CM085524	H76R	1 (0.29)	–
c.307G > A	rs2229616	CM030481	V103I	4 (1.18)	–
c.380C > T	rs13447331	CM030234	S127L	1 (0.29)	–
c.439A > G	NA	NA	R147G	1 (0.29)	–
c.449C > T	NA	CM003759	T150I	1 (0.29)	–
c.751A > C	rs52820871	CM030483	I251L	1 (0.29)	2 (1.3)
c.754G > A	rs13447336	CM990835	G252S	1 (0.29)	–
c.968G > A	NA	NA	G323E	1 (0.29)	–
Total				13 (3.8)	2 (1.3)*

AA, amino acid; HGMD, Human Gene Mutation Database; *MC4R*, melanocortin-4 receptor; *N*, number of alleles identified.
*$P < 0.05$ versus obese patients.

The maximum lifetime weights shown by the two carriers in the BN group [65 kg (BMI 24.2) and 75 kg (BMI 28.0)] were below the threshold of the WHO for obesity. At the time of the study, weights were 63.2 kg (BMI 23.5) and 68.40 kg (BMI 25.4) compared with 61.33 ± 17.91 and 23.41 ± 6.20 kg/m^2 for noncarriers. Differences were not statistically significant. Interestingly, predictions on the effect of the I251L SNP carried by these two patients indicate that the amino acid substitution would be benign (polyphen-2 score = 0.001; SIFT score = 1) and consequently it would not likely contribute toward obesity (see below for further discussion on the effect of this SNP). In line with our results, a previous study in BN patients could not find any *MC4R* mutation in individuals with normal weight; indeed, only one extremely obese BN patient out of 108 participants carried an *MC4R* mutation in that study (Hebebrand *et al.*, 2002). It should be noted that, even though obesity is a risk factor for the development of BN, the weight status is not a diagnostic criterion for BN.

The majority of the SNPs identified in our series have been subjected previously to functional analyses. For instance, an impaired MC4R response to α-MSH has been reported for some of these mutations, which would suggest a mechanism underlying the obese phenotype (Hinney *et al.*, 1999, 2013; Lubrano-Berthelier *et al.*, 2003a, 2003b). In our study, the only individual carrying two polymorphisms (S127L and G252S) was a 27-year-old woman (120 kg; BMI 43 kg/m^2), although according to Hinney *et al.* (1999), only the S127 SNP affects protein function [we have previously published an in-depth description of this patient (Albuquerque *et al.*, 2014)]. The R147G and G323E SNPs were first identified by our group very recently (Albuquerque *et al.*, 2014). Both mutations result in nonconservative amino acid substitutions, although according to polyphen-2 prediction software, the effect of the R147G would be deleterious, whereas the predicted impact for G323E is very low and therefore its contribution toward the obese phenotype is doubtful (Albuquerque *et al.*, 2014). Further predictions with SIFT (JCV Institute, La Jolla, California, USA) point to the same conclusion (R147 score = 0, damaging; G323 score = 0.15, tolerated).

The location of MC4R in the brain and the fact that alterations in its gene locus can result in hyperphagia (Huszar *et al.*, 1997) make it tempting to speculate that mutation carriers could show particular psychopathological features. To study this hypothesis, the 97 ED patients included in the study were asked to complete the EDI-2 and SCL-90R self-reported questionnaires, which have been utilized previously to identify psychopathological symptoms in patients with binge-eating episodes (Brambilla *et al.*, 2009). The two I251L carriers showed higher scores for the overall EDI-2 results (the mean value was 153.0 vs. 116.6 ± 38.1 in noncarriers) and for two scales that might be related to MC4R activity, namely, Bulimia, which measures episodes of binge-eating and purging (15.0 vs. 10.0 ± 7.2), and Interoceptive Awareness (18.0 vs. 14.1 ± 6.7 in noncarriers), which measures the ability to discriminate between sensations of hunger and satiety. In the same manner, overall SCL-90R scores were higher in the two carriers (Global Severity Index 2.25 vs. 1.93 ± 0.84). Furthermore, both women scored higher than three (usually the threshold for positive symptoms) in the Obsessive-Compulsive and Anxiety scales of the SCL-90R questionnaire. However, there are several reasons to believe that these findings are not likely to be clinically relevant.

First, differences were not statistically significant for any of the results reported (although it is obvious that the presence of only two carriers limited the statistical power). Second, both women also showed high scores for other psychopathological scales not directly associated with binge-eating (data not shown). Third, and most importantly, one could relate the high scores in the Anxiety and Obsessive-Compulsive scales to an inability to recognize satiety sensations because of a 'defective' *MC4R* gene. However, to reach this conclusion, we would have to consider what exactly the functional relevance of the I251L SNP is, an issue that has generated a great deal of controversy. For instance, Branson *et al.* (2003) identified this mutation in five obese patients with BED and reported a strong association between this disorder and other *MC4R* genetic variants. Conversely, several studies could not reproduce these results and concluded that binge-eating episodes were not characteristic of obese adult carriers of *MC4R* mutations (Hebebrand *et al.*, 2004; Lubrano-Berthelier *et al.*, 2006; Valette *et al.*, 2014). In-vitro research seems to support these negative findings because several studies have reported that the I251L SNP does not apparently impair the receptor function (Vaisse *et al.*, 2000; Nowacka-Woszuk *et al.*, 2011; Thearle *et al.*, 2012).

Several limitations have to be considered in the present study. First, the relatively small size of the population studied, particularly of the ED group, might affect the generalizability of these results. However, this limited sample also allowed for all the patients to be diagnosed and treated by the same clinicians in the same Eating Disorder Unit over a short period of time, which reduced the chance that the findings may be because of population structure. A second limitation is that a control group of individuals with normal weight was not included.

Conclusion

In summary, the results of the present pilot study have shown that functional variants in the *MC4R* gene are more commonly found in obese individuals than in nonobese individuals with binge-eating episodes. The lack of association of *MC4R* variants with binge-eating behavior had already been reported in obese patients, but we now confirm it in nonobese women diagnosed with ED. Given the low frequency of *MC4R* variants and the relatively low sample size analyzed, further studies in larger, homogenous cohorts will be needed to confirm these findings.

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Conflicts of interest

There are no conflicts of interest.

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Perspective

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Pharmacogenomics

Discussing the putative role of obesity-associated genes in the etiopathogenesis of eating disorders

In addition to the identification of mutations clearly related to Mendelian forms of obesity; genome-wide association studies and follow-up studies have in the last years pinpointed several loci associated with BMI. These genetic alterations are located in or near genes expressed in the hypothalamus that are involved in the regulation of eating behavior. Accordingly, it seems plausible that these SNPs, or others located in related genes, could also help develop aberrant conduct patterns that favor the establishment of eating disorders should other susceptibility factors or personality dimensions be present. However, and somewhat surprisingly, with few exceptions such as *BDNF*, the great majority of the genes governing these pathways remain untested in patients with anorexia nervosa, bulimia nervosa or binge-eating disorder. In the present work, we review the few existing studies, but also indications and biological concepts that point to these genes in the CNS as good candidates for association studies with eating disorder patients.

Keywords: anorexia nervosa • binge eating • BMI • bulimia nervosa • eating disorders • energy homeostasis • genetics • hypothalamus • obesity • polymorphisms

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Disorders of eating behavior (ED) have become the third most chronic disease among young females in western societies. They are associated with poor quality of life, situations of obesity or malnutrition and high rates of psychosocial morbidity and premature mortality [1]. Moreover, their treatment requires the action of a multidisciplinary team, often involving long-term hospitalization, which leads to a large consumption of resources [2].

ED are classified in three different entities according to the new edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) [3]. Anorexia nervosa (AN) is characterized by self-restriction in energy intake and obsessive fears of being fat leading to significant weight loss. Two types of AN can be differentiated: the binge-eating/purging subtype (ANBP), where patients adopt purging behaviors in addition to food restriction, and the restricting subtype (ANR), where only restraint from energy intake is present. Bulimia nervosa (BN) comprises uncontrolled episodes of binge-eating followed by compensatory conducts such as purging or extreme exercising, while binge-eating disorder (BED) is characterized by compulsive overeating without subsequent purging episodes.

ED are rare in the general population but relatively common among adolescent girls and young women. Average prevalence rates of 0.3% for AN and 1% for BN have been reported for young females [4]. In the past, ED have been characterized as culture-bound syndromes, specific to subjects from industrialized nations where food is plentiful and thinness for women is correlated with attractiveness. However, several studies have demonstrated that abnormal eating behaviors also occur in non-Western countries [5,6]. This fact has mainly been associated with globalization, modernization, urbanization and media exposure that promote the Western beauty ideals [6].

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Even though ED are low-prevalence diseases [4,7], their increasing incidence in the last two decades [8] has boosted the number of research studies focused on their etiopathogenesis. In this regard, there seem to be common etiological aspects in these disorders, as a crossover exists from AN to BN (up to 60%) and from BN to AN (less frequently) in the first years of the disease [9]. Indeed, Bulik *et al.* demonstrated an overlap of both genetic and unique environmental factors that influence the two conditions [10]. ED are multifactorial diseases, although the relative contribution of each factor is still a matter of debate [11,12]. The main susceptibility elements involved can be summarized in sociocultural reasons, especially in the western world family environment, psychological traits and biological factors, mainly endocrine and genetic features, in which we will focus on this review.

The genetic factor in eating disorders & related psychological traits

Genetic predisposition in ED is widely accepted nowadays. Estimates for the relative risk values of AN in first-degree female relatives of probands with AN or BN have been reported to be as high as 11.3 and 12.3, respectively; whereas the estimated relative risk for BN was apparently lower, approximately 4, in both cases [13]. Twin studies have been designed to differentiate between the influence of genetics and that of environment. These works confirm the heritability of AN and BN, reporting estimates between 48 and 88% for AN and 28 and 83% for BN [10,14–18]. The figures for BED seem to be lower, with a heritability ranging from 27 to 57% [19]. An interesting hypothesis is that the contribution of genetics may be different at different ages. Klump *et al.* reported the impact of genetics on eating behavior to be more important during mid-late adolescence than in prepubertal girls, which suggests that ED susceptibility genes would be activated during puberty [20].

Psychological traits that are often coupled with ED have also been shown to display heritability. For instance, two studies have shown heritability estimates for intentional weight loss in female subjects of 30–65% (just below 40% for men) [21,22]. In addition, one study has proposed a genetic contribution (43%) to restrained eating [23], although there also are negative findings in this regard [24]. The Eating Disorder Inventory [25] defines drive for thinness as an excessive concern with dieting, preoccupation with weight and entrenchment in an extreme pursuit of thinness. This trait has also been analyzed in twin studies, with heritability estimates between 45 and 50% [26,27]. Perfectionism, has in the same manner been shown to have significant additive genetic contributions [28,29]. Finally, other significant psychological characteristics such as binge-eating and self-induced vomiting have equally been observed to at least partially depend on the genetic background, with estimates for the latter trait being generally higher (up to 70%) [14,30–32].

Trying to find genetic determinants: genome-wide association studies in eating disorders

Genome-wide association studies (GWAS) have flourished in the last years as designs capable of searching the whole genome for thousands of SNPs. Despite its drawbacks, in particular the need for large samples and the high rate of false positives, GWAS have successfully identified potentially relevant loci in several psychiatric disorders [33,34].

The establishment of different consortiums has enabled large-scale sample collections, which in turn has opened the possibility of performing genome-wide analysis in ED. Three GWAS with AN cases and controls have been carried out to date. The studies by Nakabayashi *et al.* and Wang *et al.* found several variants associated with AN, but none of them reached a significance greater than $p < 10^{-5}$ [35,36] (genome-wide significance is usually set at $p < 10^{-8}$). Very recently, Boraska *et al.* have published the third and largest-to-date GWAS in AN with almost 3000 cases from 14 countries and roughly 15,000 matched controls. However, in spite of some suggestive findings, no genome-wide significance for any SNP was observed [37]. Similarly Wade *et al.* recently reported only suggestive findings in a GWAS on female twins with AN and BN [38].

In summary, GWAS aimed to locate susceptibility loci for ED have not yielded conclusive results, there are some suggestive findings but few genes stand out across the different studies published.

Candidate genes in biological pathways involved in eating disorders: the link with obesity

The classification of the biological routes with implications in eating behavior is somewhat arbitrary, depending on the function attributable to each gene, for example, dopaminergic genes are obviously part of central neurotransmission but they also have a prominent role in the management of reward processes. In any case, the main pathways involved in eating behavior could be roughly summarized in central neurotransmission, especially serotonin, dopamine and, to lesser extent, noradrenaline, whose involvement in ED has been extensively studied; reward-related pathways, particularly the cannabinoid endogenous system (it has been suggested that AN patients have a dysregulation of this

system and that restriction and exercise become a way to compensate for diminished response to reward [39]) and central regulation of food intake, including ghrelin, an appetite-stimulating hormone that functions as a neuropeptide in the CNS and whose levels may be altered in women with AN [40]. In addition, a variety of other genes that do not strictly fit in this classification have been tested in genetic association studies with ED patients, albeit with generally inconsistent results [41–49].

Several excellent reviews have been published in recent years that provide an in-depth look into the impact of genetic polymorphisms in genes involved in the aforementioned pathways [39,50–53]. However, these same authors agree in that it is striking that the majority of known genes involved in the central regulation of weight, such as those recently evidenced by GWAS and follow-up studies in obese individuals [54], have not yet been explored in association studies with ED [39,50]. In fact, fewer than 40% of the more than 100 genes [55] participating in these processes have been studied [50]. The existence of this gap in the research of the ED is especially surprising because one could easily make a compelling case for the involvement of BMI/obesity-related SNPs in BN, BED or even ANBP.

In a thought-provoking paper, Day *et al.* [56] discuss about the links between obesity and ED and how obesity is generally considered as a medical illness with metabolic and genetic origins, while ED have been traditionally regarded as Western culture-bound syndrome best treated by psychological interventions. The authors argue against this polarization and comment on the similarities in phenotype and the shared personality dimensions and risk factors. These include body dissatisfaction, low self-esteem, anxiety, depression, traits mediating substance abuse, dieting, binge-eating, a history of sexual and/or physical abuse, etc. [56]. While some of these traits are the obvious result of the disease, it is likely that the direction of causality runs both ways. Indeed, psychopathological symptoms have been shown to alter eating behavior which in turn leads to obesity [57].

We can also discuss links between ED and obesity from an evolutionary genetic perspective. The Western lifestyle surely contributes to increase obesity but it also has risk factors that may facilitate the development of ED. Could natural selection have favored the spread of genes that predispose to both these disorders? Among the several evolutionary hypotheses that have been proposed, it is interesting that both the Adaptative Viewpoint of Obesity [58] and the Adapted to Flee Famine Hypothesis in AN [59] suggest that a common need to survive hostile environments could have induced changes in genes leading to either promoting fat accumulation or food restriction, which in turn could be in the origin of evolutionary mechanisms predisposing to obesity and ED, respectively. Further supporting this hypothesis of a genetic connection between these two disorders, Bouchard *et al.* has reported a common linkage peak in chromosome 15 for both eating behaviors and susceptibility to obesity [60]. Similarly, regions at chromosome 10 have been found in genome-wide scan linkage analyses to harbor strong linkage peaks for both BMI/obesity and BN [61,62].

Obesity-associated genes in eating disorders

Following the above-mentioned rationale that argues for an intimate connection between ED and obesity, we can expect that genetic alterations in central pathways involved in the regulation of eating behavior that may lead to obesity (Figure 1), could also help develop aberrant conduct patterns that favor the establishment of ED, should other susceptibility factors or personality dimensions were present.

In the present work, we will focus on genes in the CNS whose genetic variability has been consistently associated with BMI or obesity but that, for the most part; remain untested in the field of ED. We will review the few existing data, but also indications and biological concepts, which can make these genes good candidates for association studies with ED risk or with related psychological traits. It is not the goal of this work, however, to discuss those mutations leading to monogenic forms of syndromic or nonsyndromic obesity [63], since these patients do not generally develop ED.

Brain-derived neurotrophic factor

The brain-derived neurotrophic factor (BDNF) is by far the best studied obesity-associated gene in relation to ED. BDNF is a neurotrophin initially synthesized as a precursor, which is then processed leading to the mature BDNF protein. BDNF then binds to the tropomiosin kinase receptor type B (TrkB), thus triggering receptor dimerization, autophosphorylation and subsequent activation of a signaling cascade [64].

BDNF plays a key role in the early stages of embryogenesis, promoting the differentiation of precursor stem cells of the CNS. In the later stages of development, BDNF regulates cortical dendritic growth, branching and remodeling of the optic nerve, the development of dopaminergic connections and hippocampal neurons [65,66].

BDNF is essential for body weight control and energy homeostasis. Animal studies in knockout models have shown hyperphagia, hyperactivity, anxiety and weight gain [65,67,68]. Furthermore, BDNF haploinsufficiency in humans is also associated with decreased satiety, hyperphagia and obesity [69]. There

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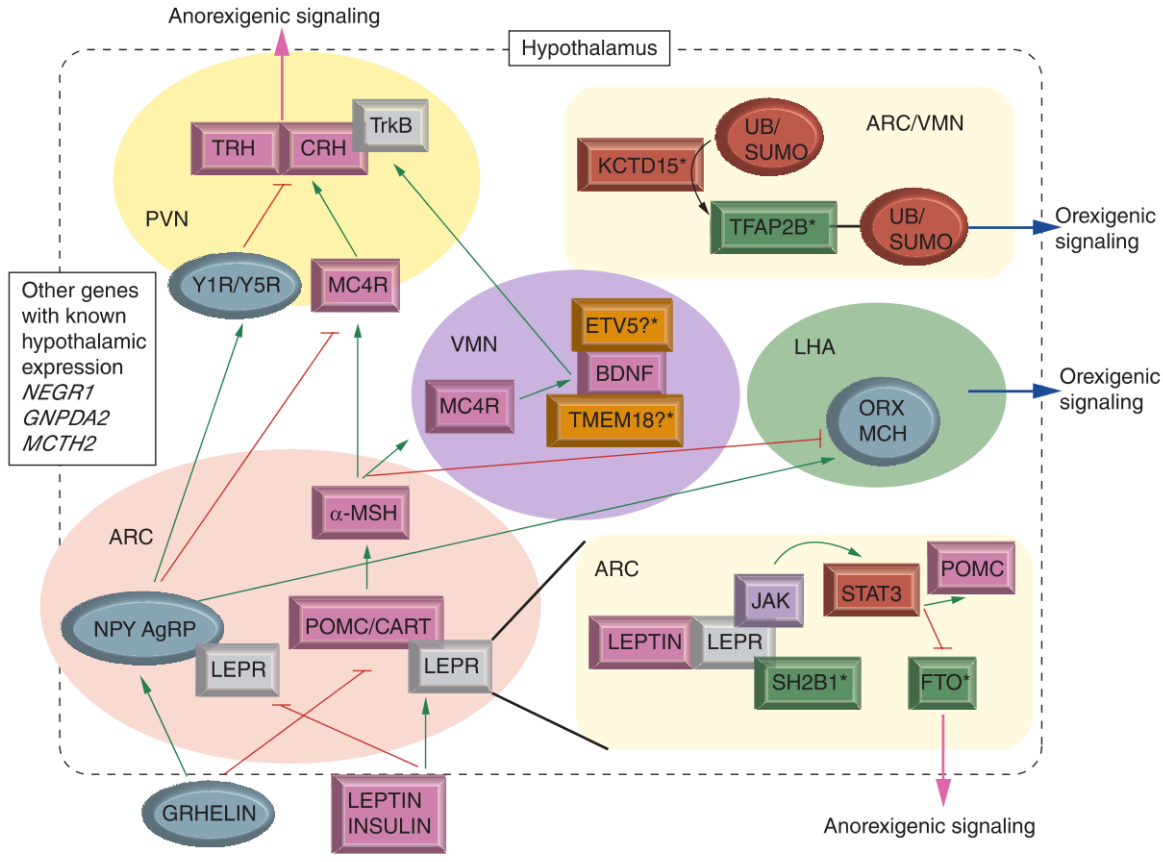

Figure 1. Regulation of food intake in the hypothalamus. Leptin, secreted by adipocytes, is transported across the blood–brain barrier to act on ARC. In this location, leptin exerts opposite effects on the activity of POMC/CART and NPY/AgRP neurons. POMC/CART neurons release α-MSH, which binds to MC4R in VMN and PVN. In VMN, BDNF acts as an effector within the MC4R pathway. It is not clear but ETV5 and TMEM18 could also interact with BDNF in this step. In addition, BDNF regulates CRH signaling through TrkB in PVN. In this same location, α-MSH binds to MC4R and activates TRH and CRH expression, which leads to an anorexigenic state. Additionally, in response to high leptin concentrations, the LEPR-JAK-SHB2-STAT3 signaling pathway could result in FTO downregulation. Alternatively, if no leptin is present or there is an increase of grhelin levels, the orexigenic peptides NPY and AgRP are expressed. AgRP is a direct antagonist of MC4R and stimulates ORX and MCH expression in LHA, which leads to an orexigenic state. NPY acts on PVN through Y1R and Y5R receptors, thus inhibiting the expression of TRH and CRH. TFAP2B and KTCD15 co-localize in ARC and VMN, here it is possible that KCTD15 acts like a scaffold where TFAP2B is either SUMOylated or ubiquitinated, which would lead to consummatory behavior.
*Genes that have been identified as obesity loci in genome-wide association studies. Color code: Blue, orexigenic pathways; Pink, anorexigenic pathways; Green, activation; Red, inhibition.
AgRP: Agouti-related protein; ARC: Arcuate nucleus; BDNF: Brain-derived neurotrophic factor; CART: Cocaine- and amphetamine-regulated transcript; CRH: Corticotropin releasing hormone; ETV5: Ets variant 5; FTO: Fat mass and obesity associated; GNDPA2: Glucosamine-6-phosphate deaminase 2; JAK: Janus kinase; KCTD15: Potassium channel tetramerization domain-containing 15; LEPR: Leptin receptor; LHA: Lateral hypothalamus area; MC4R: Melanocortin 4 receptor; MCH: Melanin-concentrating hormone; MCTH2: Mitocondrial carrier homolog 2; NEGR1: Neuronal growth regulator 1; NPY: Neuropeptide Y; ORX: Orexin; POMC: Pro-opiomelanocortin; PVN: Paraventricular nucleus; SH2B: Src-homology 2 domain-containing protein B1; STAT3: Signal transducer and activator of transcription 3; SUMO: Sumoylation; TFAP2B: Transcription factor A-2 beta; TMEM18: Transmembrane protein 18; TRH: Tyrotropin releasing hormone; UB: Ubiquitination; VMN: Ventromedial nucleus; Y1R, Y5R: Neuropeptide Y receptor 1 and 5; α-MSH: α-melanocyte stimulating hormone.

is also an increasing body of evidence that highlights the important role of BDNF in feeding behavior and cognitive impairment [65,66,70–73]. All these data have prompted the study of BNDF in patients with ED. In this regard, various publications demonstrate the existence of decreased BDNF serum levels in patients with AN and BN [74–77]. Furthermore, BDNF circulating concentrations have also been shown to correlate with various psychopathological traits in patients with ED [78–80].

It has been shown that *BDNF* variants may modulate protein levels which in turn could affect the susceptibility to ED [81]. The two most commonly analyzed polymorphisms in this field are rs6252 (Val66Met) and rs56164415 (C-270T). The Val66Met SNP is located in the 5'-proregion and affects intracellular processing and secretion of BDNF [82]. This SNP has been associated with episodes of mental impairment [82] and increased susceptibility to neuropsychiatric disorders such as Alzheimer's [83] and Parkinson's disease [84], depression [85] or bipolar disorder [86]. In addition, genome-wide [87] and cohort studies [88,89] have linked this polymorphism with obesity/BMI.

The analysis of the role of the Val66Met polymorphism on ED risk has produced conflicting results (Table 1), although the most recent studies and a meta-analysis by Brandys *et al.* published in 2013 [90] seem to suggest that the impact of this SNP may not be as relevant as initially thought. The disorder most commonly targeted in these studies was AN, and the reason for the reported discrepancies might be that not all the authors stratified into the ANR and ANBP subtypes. Indeed, in the early study by Ribases *et al.* [91] that indicated a positive association, this was only observed for the risk of ANR.

The other common *BDNF* polymorphism studied in relation to psychiatric disorders is a C to T transition in the promoter region of the gene, which could affect protein expression [92]. Numerous studies have been carried out to assess the association of this SNP with several aspects of psychiatric disorders, such as schizophrenia [93,94], Alzheimer's [92,95], Parkinson's [95] or Huntington's disease [96], but these works have mostly produced negative or at least inconsistent results. In comparison with the case of the Val66Met SNP, there are fewer studies on the role of the C-270T polymorphism in ED. In two early reports by Ribases *et al.*, there was no evidence of any effect on ED risk, although a marginal association with a late age of onset of weight loss was found [91,97]. Later studies seem to corroborate these initial negative findings (Table 1).

On the other hand, given the role of BDNF in the brain and its modulation of neurotransmitters systems that may be altered in psychiatric disorders [98–101], it may be that variants in the gene locus could affect certain personality dimensions, which could in turn draw an already vulnerable patient (e.g., by familiar or environmental factors) to an ED or lead, for instance, to an aggravation of associated psychopathological symptoms. Thus, *BDNF* allelic variants have been associated in non-ED patients with personality traits such as neuroticism [85], introversion [102] and several related anxiety [103] and depression [104,105] patterns. However, very few studies have followed this design in the ED setting. Rybakowski *et al.* could not find an association between the Val66Met and temperamental traits in AN [106], which is consistent with the findings reported recently by our group in AN and BN using a different questionnaire, the Eating Disorder Inventory -2 (EDI-2) [107]. In contrast, Ando *et al.* did observe significant lower harm avoidance scores in AN patients who were Met66 carriers, although there were no differences for novelty seeking, reward dependence or persistence [108]. With regard to the C-270T SNP, the T allele has been shown to correlate with higher persistence and harm avoidance scores in AN patients [106].

The screening of the *BDNF* gene variability using tag-SNPs and haplotype analysis must be more revealing than the study of point mutations. In this regard, we recently performed haplotype analyses using a sliding window approach with three adjacent SNPs which produced four loci of interest. One of these 3-SNP combinations, that included the C-270T SNP (rs10835210/rs16917237/C-270T), showed a profound effect on personality dimensions of both AN and BN patients, as measured by the Symptom Checklist-90-Revised (SCL-90R) inventory [107]. This questionnaire is used to evaluate general psychopathological symptoms. This, along with the fact that psychological traits more intimately related to ED (measured with EDI-2) were largely unaffected in this study, seems to suggest that variability in the *BDNF* gene locus may contribute to psychopathological features that are commonly found in ED patients but that could be indicative of comorbid disorders [107].

Melanocortin-4 receptor

The melanocortin-4 receptor (MC4R) is a seven transmembrane domain receptor expressed in regions of thalamus, hypothalamus and hippocampus that activate adenylate cyclase in response to the α-melanocyte-stimulating hormone, thus mediating satiety signals [109,110]. Interestingly, it has been shown that MC4R signaling controls BDNF expression in the hypothalamus, supporting the hypothesis that BDNF is an important effector through which MC4R controls energy balance [66] (Figure 1). Inactivation of this receptor by gene targeting results in mice that develop a maturity onset obesity associated with hyperphagia [111]. Later, two independent studies described two different frameshift mutations in the gene that were associated with hyperphagia and constant search for food [112,113]. Since then, this gene has been the focus of numerous association studies aimed to identify genetic determinants of obesity. Indeed, *MC4R* mutations constitute today the most common monogenic cause of human obesity, with up to 4% of morbid obesity cases attributable to mutations in this gene [114,115].

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Table 1. Studies analyzing the role of *BDNF* Val66Met and C-270T polymorphisms in eating disorders.

Study	SNP	Disorder, n	Main findings	Ref.
Ribases *et al.*	Val66Met	ANR = 26; ANP = 36; BN = 70; Controls = 112	Met allele was associated with ANR. Lowest BMI values in Met carriers	[91]
Ribases *et al.*	Val66Met	European multicentric study. AN = 753; BN = 389; Controls = 510	Met allele associated with all ED subtypes	[97]
Koizumi *et al.*	Val66Met	AN = 97; BN = 101; Controls = 222	Global association with ED and with BN in particular	[188]
Friedel *et al.*	Val66Met	AN = 118; BN = 80; Controls = 96	No association with ED risk	[189]
Rybakowski *et al.*	Val66Met	AN = 149; Controls = 100	No association with AN risk or temperamental traits	[106]
Ando *et al.*	Val66Met	AN = 689; Controls = 573	No association with AN risk. The Met allele correlated with scores in *harm avoidance*, no relation with other personality dimensions	[108]
Brandys *et al.*	Val66Met	Case-control study and meta-analysis; AN = 235; Controls = 643	No association with AN risk. A meta-analysis was also performed with equally negative results	[90]
de Krom *et al.*	Val66Met	AN = 195; Controls = 580	No association with AN risk	[190]
Gamero-Villarroel *et al.*	Val66Met	AN = 106; BN = 63; Controls = 312	No association with the risk for ED. No relation with ED-related psychological traits	[107]
Yilmaz *et al.*	Val66Met	AN = 745, BN = 245, Controls = 321	No association with AN risk or BMI	[126]
Ribases *et al.*	C-270T	ANR = 26; ANP = 36; BN = 70; Controls = 112	No association with any ED risk	[91]
Ribases *et al.*	C-270T	AN = 753; BN = 389; Controls = 510	No association with ED risk. The C-allele was associated with a late age of onset of weight loss	[97]
Friedel *et al.*	C-270T	AN = 118; BN = 80; Controls = 96	No association with ED risk	[189]
Rybakowski *et al.*	C-270T	AN = 149; Controls = 100	No association with AN risk. The T-allele was associated with higher harm avoidance scores	[106]
de Krom *et al.*	C-270T	AN = 195; Controls = 580	No association with AN risk	[190]
Gamero-Villarroel *et al.*	C-270T (as part of a 3-SNP haplotype)	AN = 106; BN = 63; Controls = 312	No association with AN or BN risk. The rs10835210/rs16917237/C-270T was related to profound changes in personality dimensions of both AN and BN patients	[107]

AN: Anorexia nervosa; BN: Bulimia nervosa; ED: Eating disorder.

Hebebrand *et al.* first hypothesized that obesity-predisposing haplo-insufficiency mutations in the *MC4R* gene might be also frequent in BN patients with binge eating. [116]; albeit it should be mentioned that their conclusions were based on results from a single extremely obese patient with BN. The same research group had previously reported the first observation of BED associated with *MC4R* genetic alterations [117]. However, since these initial works, later studies have showed contradictory results regarding the association between binge-eating behavior and *MC4R* mutations. One research group in two different studies with obese subjects reported higher frequency of BED among *MC4R* mutation carriers compared with noncarriers [118,119], although these researchers did not consider the effect of the mutations (loss/gain of function or negligible), which represents a major limitation of their conclusions. Indeed, later reports have been unable to reproduce such association in obese individuals [120–124]. Furthermore, very recently we and others have expanded these negative findings to nonobese patients with AN or BN [125,126]. In addition, BMI-related *MC4R* SNPs have also been related with certain kind of feeding behaviors in the general population [124]. The lack of uniformity in diagnostic criteria used for evaluation of binge eating behavior, namely *ad libitum* test meals, semiquantitative interviews, questionnaires or single questions, in the different study populations

has most likely contributed to the disparity in the results [123].

On the other hand, Brandys *et al.* could not find an association of two previously BMI-related *MC4R* variants, rs17782313 and rs17700633, with AN risk [127]. However, once again there are evidences that these polymorphisms may be able to modulate personality traits that could lead to, aggravate or help maintain ED. Thus, Horstmann *et al.* recently reported that women who were homozygous carriers of the rs17782313-C allele showed a significant increase of gray matter volume in the right amygdala, a region known to influence eating behavior. These changes translated into elevated scores of disinhibition, and emotional eating. Interestingly enough, no brain structural changes associated with the SNP were found in the male participants [128].

A summary of the main findings reported by genetic association studies on *MC4R* in the ED setting are summarized in Table 2.

Fat mass & obesity-associated gene

The fat mass and obesity-associated (*FTO*) gene encodes a protein that functions as an AlkB-like DNA/RNA demethylase [129]. FTO is also expressed in dopaminergic neurons where its inactivation has been shown to impair dopamine receptor-dependent control of neuronal activity and behavioral responses [130]. Therefore, given its location and function, *FTO* genetic variants hold the potential to affect feeding-related reward processes. Indeed, several SNPs within a region in intron 1 have been robustly associated with obesity [131–134]. Null *Fto* alleles in mice result in a significant reduction in adipose tissue and lean body mass [135], whereas models overexpressing FTO display increased food intake leading to obesity [136]. In particular, the obesity predisposing risk A-allele at rs9939609 has become one of the most consistent risk factors for polygenetic obesity [137]. In a multicentric European study Müller *et al.* [138] reported a positive association for this SNP with both AN and BN, albeit odds ratios and p-values (especially for BN) were not impressive. The authors did not analyze other SNPs in or near *FTO*. In contrast, a previous study by Brandys *et al.* failed to find an association between another obesity-related *FTO* SNP, rs1121980 and AN [127]. Similarly, in a large sample of over 1000 AN patients and 677 controls, Jonassaint, *et al.* could not confirm a relation between seven polymorphisms of the *FTO* gene and the risk for AN, nor they observed any association with ED-related personality traits [139]. Finally, one large European study could not find an association of the BMI-related rs1421085 SNP and feeding behavior in the general population [124]. See Table 3 for a summary of studies on FTO SNPs.

Obesity has been suggested to be a risk factor for BN [140], accordingly, it has been proposed that obesity risk alleles may be more frequent in patients with BN [116,125]. This rationale provides with a reasonable hypothesis for the study of the association between obesity-related SNPs of the *FTO* gene and BN. However, to our knowledge only one study [138] has addressed this question in BN patients (with positive results).

An additional problem regarding genetic association studies with *FTO* in ED is that the association with obesity may likely results from several functional consequences. For instance, Smemo *et al.* [141] and Stratigopoulos *et al.* [142] have shown that the expression of both *IRX3* and *RPGRIP1L* genes is regulated by obesity-linked *FTO* SNPs. Furthermore, the intron 1 polymorphic region has been claimed to modulate the level of methylation of *FTO* but also of other genes [143].

Neuronal growth regulator 1

The neuronal growth regulator 1 (*NEGR1*) gene codes for a cell adhesion molecule of the immunoglobulin (Ig) superfamily that belongs to the IgLON subgroup and plays a key role in neural development [144]. It has been reported that loss-of-function mutations lead to inhibition of cell adhesion and neurite growth as well as to a decreased body mass in mice [145]. Furthermore, *NEGR1* variants have been associated with higher BMI in several GWAS aimed to identify obesity loci [146,147].

With regard to its role in ED, Brandys *et al.* analyzed the effect of *NEGR1* rs2568958 on AN risk, without finding evidence for this association [127], which is in line with our results in AN and BN patients [148]. However, in this last study, we also performed a combined analysis of 21 tag-SNPs of *NEGR1* in relation to a variety personality dimensions in the patients. Interestingly, haplotype analyses and a sliding-window approach identified a central region of the gene spanning 210 kb whose variability strongly correlated with higher scores of BN patients in drive for thinness, ineffectiveness and interoceptive awareness [148].

The biological mechanism by which *NEGR1* genetic variants could affect these personality traits has recently been hypothesized. Dennis *et al.* [149] just published a paper in which they show how variability in the *NEGR1* gene locus, and particularly the BMI-associated rs2815752-A allele, was related to lower white matter integrity across a substantial portion of the brain, which has been associated to a variety of psychiatric diseases. As this study was performed on healthy individuals, the authors propose that this and other BMI-related SNPs could affect the brain in ways not mediated by obesity. In other words, they could have a direct effect on the brain by influencing motivation/personality [149]. We believe the same theory

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Table 2. Summary of studies analyzing the role of *MC4R* genetic variants in eating disorders.

Study	SNP	Disorder, n	Main findings	Ref.
Hebebrand *et al.*.	Tyr35Stop; Asp37Val	BN = 81	One obese BN patient carried functionally relevant mutations, suggestive of increased predisposition to binge eating	[116]
Brandys *et al.*	rs17700633; rs17782313	AN = 267, Controls = 1636	No association with AN risk or BMI	[127]
Yilmaz *et al.*	rs17782313	AN = 745, BN = 245, Controls = 321	No association with AN risk or BMI	[126]
Branson *et al.*	Mutation screening by sequencing	OB = 469, Controls = 25	All mutations carriers reported binge-eating behavior	[118]
Hebebrand *et al.*	Screening by SSCP	OB = 1041	No association of *MC4R* mutations with binge-eating behavior after ruling out nonfunctional polymorphisms	[121]
Potoczna *et al.*	Mutation screening by sequencing	OB = 300	All *MC4R* variants carriers presented with BED. They lost less weight after laparoscopic gastric banding, with more complications and overall poorer outcome than noncarriers with BED	[119]
Lubrano-Berthelier *et al.*	Mutation screening by sequencing	OB = 769, Controls = 444	Obese carriers of *MC4R* mutations did not present with binge eating or any other specific phenotype	[122]
Valette *et al.*	Mutation screening by sequencing	OB = 4653	Functional *MC4R* mutations were not associated to a higher risk of compulsive eating	[123]
Gamero-Villarroel *et al.*	Mutation screening by sequencing	Non obese BN/BED = 77; OB = 170; AN = 20	Mutations in the *MC4R* gene were not associated with binge-eating behavior in nonobese ED patients	[125]
Stutzmann *et al.*	rs17782313-C allele	OB = 2383; Controls = 10489	Higher incidence of snacking, higher Stunkard hunger score and higher prevalence of eating large amounts of food	[124]
Horstmann *et al.*	rs17782313-C allele		Sex-specific (females) increase of gray matter volume. Higher scores of disinhibition and emotional eating in women who were homozygous carriers	[128]

AN: Anorexia nervosa; BED: Binge-eating disorder; BN: Bulimia nervosa; ED: Eating disorder; OB: Obese.

could be formulated for the involvement of *NEGR1* polymorphisms in ED.

Transcription factor AP-2 beta

The transcription factor AP-2 beta (*TFAP2B*) gene belongs to the AP-2 family of transcription factors, which are critical for the regulation of neural development and gene expression in the CNS, being preferentially expressed in hippocampus, midbrain and cerebral cortex [150]. Several dopaminergic and serotonergic genes in the midbrain have AP-2 binding sites; therefore it is likely that this transcription factors, aside from its established role during development, can also influence mood and personality in adults through this neurotransmitter system [150].

Most notably, *TFAP2B* contains a polymorphic region which includes a four-nucleotide repeat [CAAA] of four- of five-times located in intron 2 [151]. This genotype has been shown to be associated with personality dimensions such as anxiety, psychasthenia, depression or antisocial traits, with the short allele generally correlating with lower scores in the different measured psychological scales [152–155]. Furthermore, and supporting the aforementioned biological function of *TFAP2B*, this polymorphism has been suggested to interact with polymorphic sites in the serotonin transporter (*5HTTLPR*), catechol-O-methyl transferase (*COMT*) and monoamino oxidase (*MAO*)-*A* genes to modulate additional personality dimensions [154,156].

To our knowledge, there is only one study that assesses the role of the $[CAAA]_{4-5}$ genotype in ED patients. Damberg *et al.* analyzed a small sample of 46 obese women with BED and 76 healthy female volunteers, and reported a higher frequency of homozygous subjects for the long allele in patients with BED compared with control subjects [150]. The authors also provide with a hypothesis for the impact of this polymorphism on eating behavior. Their data indicate that the $[CAAA]_5$ homozygous genotype was also linked to low thrombocyte monoamine oxidase (trbcMAO) activity,

a B-type MAO with the same amino acid sequence as brain MAO-B. These low levels of trbcMAO have been shown to correlate with personality characteristics such as sensation seeking or impulsiveness, which are generally present in BED patients [157].

Src-homology 2 domain-containing protein B1

The Src homology 2B (SH2B) family members (SH2B1, 2 and 3) are adaptor signaling proteins containing characteristic SH2 and PH domains. The C-terminal SH2 domain of SH2B1 binds to tyrosyl phosphorylated proteins to enhance leptin and insulin signaling. SH2B1 also participates in the downregulation of FTO through STAT3 signaling in the hypothalamus (See Figure 1). Genetic deletion of *SH2B1* results in severe leptin resistance, insulin resistance, hyperphagia and obesity [158]. Notably, the obesity in Sh2b1-null mice can be reversed by targeted Sh2b1 expression in neurons, suggesting that the effects of this gene in obesity are mediated through the CNS [159].

SH2B1 has also been suggested to modulate psychopathological traits. Thus, Doche *et al.* linked the presence of loss-of-function mutations in the gene with a spectrum of behavioral abnormalities in obese subjects including social isolation and aggression [160].

Rs7498665 in *SH2B1* is one of the SNPs initially identified in GWAS with significant linkage to BMI whose association has been confirmed by follow-up studies using computed tomography as a measure of adiposity [161]. With regard to its involvement in ED, and as in the case of the other BMI-related polymorphisms, Brandys *et al.* could not find an association of rs7498665 with AN risk [127]. Only a marginal evidence of association between this SNP and snacking behavior has been reported for the general population [162].

Transmembrane protein 18

Several GWAS studies have confirmed the existence of a BMI/obesity-linked locus in the transmembrane protein 18 (*TMEM18*) gene [147,163,164], whose effect could also be mediated through interaction with BDNF [165]. *TMEM18* codes for a transmembrane protein expressed in several regions of the brain, including the hypothalamus, where its expression would translate into feeding behavior [166,167]. Although its specific function is yet to be established, TMEM18 may be involved in cell migration, by regulating neuronal stem cell mobility through transcription repressing mechanisms [166,168]. In spite of its involvement in obesity through the modulation of feeding behavior [166], this gene has only been assessed in one study with AN patients that reported no association with the risk for the disease for the BMI-linked rs6548238 SNP [127]. Additional studies that could evaluate the influence of *TMEM18* genetic variants on personality dimensions in ED patients could help elucidate its role in the regulation of eating behavior. Moreover, given the location and role of *TMEM18*, it would be interesting to analyze the status of this gene in individuals with a binge-eating component.

Glucosamine-6-phosphate deaminase 2

The protein encoded by the glucosamine-6-phosphate deaminase 2 (*GNPDA2*) gene is an allosteric enzyme that catalyzes the reversible reaction converting D-glucosamine-6-phosphate into D-fructose-6-phosphate and ammonium.

The rs10938397 polymorphism near the *GNPDA2* gene has been identified as an obesity locus in GWAS [147]. Interestingly, two subsequent studies have related this SNP to sedentary behavior [169] and attention-deficit/hyperactivity disorder [170], thus establishing a link between this gene and conduct. In addition, genetic variability in this locus has also been associated with vigorous physical activity in adolescents, a feature also present in some ED patients [171]. Rask-Andersen *et al.* in 2010 pointed out this gene as an untested locus of interest for the study of AN [50]. The same year, the rs10938397 SNP was tested by Brandys *et al.* in the case-control study in AN to which we have made reference repeatedly, but again there was no association with the risk for the disease [127]. However, *GNPDA2* could be a good candidate gene for studies in patients with ED other than AN, in other words, BN and BED, or in studies analyzing ED-related personality dimensions.

Mitochondrial carrier homolog 2

Mitochondrial carrier homolog 2 (MTCH2) is a conserved protein that belongs to the mitochondrial carrier protein family, which catalyze the exchange of solutes across the inner mitochondrial membrane and that may play an important role in cell apoptosis [172,173]. MCTH2 is expressed in several locations including the hypothalamus, a crucial center for regulation of food intake and energy homeostasis [174,175].

One of the recently identified obesity-associated SNPs in GWAS, rs10838738, is close to the *MTHC2* gene [146,147]. This SNP could not be related to AN risk [127], although it has been linked to eating behavior in healthy subjects [174]. In a later study, Cornelis *et al.* interviewed 3852 individuals using the Three Factor Eating Questionnaire (TFEQ)-R18 and genotyped the participants for SNPs in genes expressed in regions of the brain that regulate energy intake and reward-seeking behavior. The authors found that the rs3817334 of the *MTCH2* gene was positively associated with emotional eating, reflecting lack of homeostatic control or greater

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Table 3. Studies analyzing the impact of *FTO* obesity-related polymorphisms on eating disorders.

Study	SNP	Disorder, n	Main findings	Ref.
Brandys *et al.*	rs1121980	AN = 225; Controls = 1636	No association with AN risk	[127]
Jonassaint *et al.*	rs7193144; rs8043757; rs3751812; rs11075990; rs9941349; rs17817964; rs9930506	AN = 1085; Controls = 677	No association with AN risk or with ED-related personality traits	[139]
Muller *et al.*	rs9939609	Multicentric European study: AN = 689; BN = 477; Controls = 4935	The A-allele was associated with both AN and BN	[138]
Stutzmann *et al.*	rs1421085C-allele	OB = 2383; Controls = 10489	No association with eating behavior traits	[124]

AN: Anorexia nervosa; BN: Bulimia nervosa; ED: Eating disorder; OB: Obese.

sensitivity to food reward feedback [176]. These findings indicate that variability in the *MTCH2* gene locus may be related to eating behavior, which in turn could be of relevance not only for obesity but also for the ED field.

ETV5 transcription factor

ETV5 (also known as ERM) belongs to the ETS family of transcription factors, which are ubiquitously expressed and participate in the activation of genes that are central to tumor invasion and angiogenesis through the regulation of cellular proliferation, differentiation and apoptosis [177].

Even though GWAS have revealed an association of SNPs near *ETV5* with obesity [147,178], the specific role of ETV5 in regulating energy homeostasis is yet to be established. There are some indications, however, that its participation could be significant. For instance, Etv5-/- mice have been shown to have reduced body weights compared with wild-type controls [179]. In addition, the nutritional state has been shown in animal models to affect the expression of Etv5 in important areas for feeding behavior [180], although this overexpression did not translate into significant changes of eating habits [181]. In any case, it has been suggested that, being a transcription factor, the possibility that ETV5 influences energy balance by transcriptional regulation of key genes linked to food intake in the hypothalamus seems highly likely [161]. Interestingly, BDNF has been shown to have a principal role orchestrating ETV5-mediated cellular migration [182].

Other than the study by Brandys *et al.*, which reported the lack of association for *ETV5* rs7647305 with AN risk, there are no data on the role of *ETV5* variants in ED.

Potassium channel tetramerization domain-containing 15

The potassium channel tetramerization domain-containing 15 (*KCTD15*) gene interacts with the above-mentioned AP-2 family of transcription factors, repressing their transcriptional activity, and eventually delimiting neural crest formation in order to prevent its lateral expression [183]. Extensive population-based GWAS [147,178,184] have identified SNPs in or near the *KCTD15* gene as putative regulators of BMI. One of these variants (rs368794) has been assessed in relation to AN risk but no association was observed [127].

Conclusion & future perspective

Among the great amount of genes that have been proposed as suitable candidates for genetic association studies in ED, of special interest are those involved, directly or indirectly, in the molecular mechanisms of control of food intake and body weight in the hypothalamus [50,51,185]. Several of these loci have already been consistently associated with obesity through GWAS and replication studies (reviewed by Waalen *et al.* [54]). The hypothesis for this association is that the modulation of eating-related behavior conferred by polymorphisms in these genes may lead to changes in the BMI and subsequent obesity. This has been recently demonstrated by Cornelis *et al.*, who showed that a genetic-risk score derived from 32 obesity-loci, including those reviewed in the present work, was positively associated with emotional and uncontrolled eating. The authors concluded that eating behaviors may contribute significantly to the link between genetic variation and the development of obesity and motivate future investigations on the role of these loci [176]. Therefore, it seems evident that these genes should automatically be the target of association studies in ED. As we mentioned earlier, AN might not be the ideal disorder to test these genes in the first place. This is reflected by the negative findings reported in a study that assessed the putative role of six GWAS-identified obesity loci in the susceptibility to AN. The authors suggested that effects of those variants may be overridden by other factors of susceptibility [127].

Table 4. Studies analyzing the effect of other BMI-related polymorphisms of central genes in eating disorders.

Study	Gene, polymorphism	Disorder, n	Main findings	Ref.
Damberg *et al.*	*TFAP2B*, 4-nucleotide repeat in intron 2	BED = 46; Controls = 73	The long-allele homozygous genotype was more frequent among BED patients	[150]
Gamero-Villarroel *et al.*	*NEGR1*, 21 tag-SNPs	AN = 106; BN = 63; Controls = 312	No association with the risk of AN or BN Haplotype analyses identified a central area of the gene whose variability correlated with personality dimensions scores in BN patients	[107]
Brandys *et al.*	*NEGR1*, rs2568958	AN = 267; Controls = 1636	No association with the risk for the disease	[127]
Brandys *et al.*	*KCTD15*, rs368794	AN = 267; Controls = 1636	No association with the risk for the disease	[127]
Robiou-du-Pont *et al.*	*SH2B1*, rs7498665	Snackers = 1868; Controls = 5634	Association with snacking behavior	[162]
Brandys *et al.*	*SH2B1*, rs7498665	AN = 267; Controls = 1636	No association with the risk for the disease	[127]
Brandys *et al.*	*GNPDA2*, rs10938397	AN = 267; Controls = 1636	No association with the risk for the disease	[127]
Cornelis *et al.*	MTHC2, rs3817334	3852 healthy men and women	Positive association with emotional eating	[176]
Brandys *et al.*	*MTCH2*, rs10838738	AN = 267; Controls = 1636	No association with the risk for the disease	[127]
Brandys *et al.*	*ETV5*, rs7647305	AN = 267; Controls = 1636	No association with the risk for the disease	[127]

AN: Anorexia nervosa; BED: Binge-eating disorder; BN: Bulimia nervosa; ED: Eating disorder.

We have seen that these obesity/BMI-related variants are also related to impulsiveness, eating great amount of foods, increases in hunger score, snacking, etc., and these are behaviors that one could more easily relate to BN or BED. Paradoxically, and probably with the exception of *BDNF*, the vast majority of available genetic association studies on the regulation of appetite and energy homeostasis have been carried out in AN. Furthermore, diagnoses in these works are not frequently stratified into restricting- and binge-purging subtypes. In general, there is a need for more studies in BN or BED patients that can examine these central pathways, either with GWA or with candidate-gene approaches. Efforts should be put into collecting large samples from BN and BED patients, as it is happening for instance with the Genetic Consortium for AN (GCAN). In this regard, Boraska *et al.* recently performed the largest-to-date GWAS study on this dataset [37], which could also be useful to specifically assess the effect of all identified SNPs related to BMI, weight or obesity.

We may argue, however, that expanding the sample size to genome-wide necessities also implies a decrease in the homogeneity of the study designs. While diagnosis for AN or BN are generally well established, we find significant differences with regard to the definition of binge-eating behavior across studies. Hopefully the newly implemented DSM-V, which categorizes BED as a whole different entity at the same level as AN or BN, may improve the consistency of the diagnosis. The lack of homogeneity is especially worrying when it comes to large, international studies that assess personality dimensions in ED patients. The fact that these data are collected mostly through personal questionnaires makes it difficult to compare information on patients that are interviewed by clinicians in different countries with different idiosyncrasies. In this regard, it has also been suggested that measuring *ad libitum* food intake provides with highly reproducible information, which is more accurate than self-reporting [186]. However, this methodology is likely more difficult to implement in clinical routine, particularly in studies with outpatients, where the use of self-reported questionnaires is widespread.

Overall, and as difficult as it is, we should try to find a balance between sample size and homogeneity, especially in studies measuring scores in psychological traits.

Another concern we have to bear in mind is the extrapolations that are made from GWAS findings in ED patients. As we have described before for the case of the association between the SNPs near *FTO* and obesity [141], caution should be exerted when interpreting a GWAS hit

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that lies in no man's land. In this regard Tung *et al.* rightly raised a concern in a recent opinion paper: *"just sticking a pin in the nearest coding sequence to a GWAS hit and studying the consequence of loss of that gene is maybe a too simple way to proceed. It is imperative to move from GWAS signals to biological understanding"*[187]. We fully agree with that statement; even though GWAS are powerful tools that allow the study of a great amount of genes and SNPs, we should find a way to combine this great capability with the existence of a hypothesis that may, at least remotely, support further analysis of the target genes (Table 4).

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Executive summary

Genetic factor in eating disorders

- Family and twin studies have repeatedly shown that genetics play a significant role in the susceptibility to eating disorders (ED) as well as in associated psychological traits. However, no individual gene has as yet been pointed out as a true determinant of these diseases.
- Three genome-wide association studies (GWAS) in anorexia nervosa (AN) carried out to date have only revealed suggestive findings.

Candidate genes in biological pathways involved in ED

- Three main biological routes have been explored in relation to eating disorders, namely, central neurotransmission, reward processes and central regulation of food intake and energy homeostasis.
- Out of these, the central regulation of food intake, which takes place mainly in the hypothalamus, is by far the less studied in relation to ED. We believe this is an important gap in the research of ED genetics.

Link with obesity

- Obesity is a risk factor for bulimia nervosa (BN) and a significant proportion of people with obesity meet the criteria for binge eating. There are also a variety of psychological traits that are shared between these diseases.
- Several GWAS and follow-up studies have consistently identified a number of SNPs in central genes that are linked to BMI and/or obesity. We and others hypothesize that these SNPs could also help develop aberrant behavior patterns that favor the establishment of ED or affect associated personality dimensions.

Evidences that relate energy-homeostasis regulating genes to disordered eating

Brain-derived neurotrophic factor

- The rs6252 (Val66Met) polymorphism is the most studied SNP in this gene but in spite of some early promising results, especially in ANR, later research has been more cautious regarding its role in ED.
- Although the effect on the susceptibility to ED is doubtful, variability in the *BDNF* gene locus may contribute to psychopathological features that are commonly found in ED patients and could be indicative of comorbid disorders.

Melanocortin-4 receptor

- The lack of uniformity in the diagnostic criteria of binge eating behavior could contribute to the disparity in the results reported with regard to the involvement of *MC4R* SNPs in this disorder.
- SNPs in *MC4R* are associated with structural changes in regions of the brain known to influence eating behavior. Furthermore, these changes translate into increased disinhibition and emotional eating.

Fat mass & obesity-associated gene

- *FTO* SNPs have been robustly associated with obesity but its involvement in AN or related personality traits could not be confirmed. However, the only study in BN patients found a positive association.
- A recent report raised the concern that obesity-associated, GWAS-identified SNPs near *FTO* may in fact affect the expression of other genes, for example, *IRX3*.

Neuronal growth regulator 1

- Our group has recently identified a central area of this gene, spanning 210 kb, which strongly correlates with several psychological traits in BN patients.

Transcription factor AP-2 beta

- One report has showed an association of a four-nucleotide repeat polymorphism with binge eating. The study also provided a biological hypothesis, involving the MAO enzyme, for the impact of this polymorphism on eating behavior.

Src-homology 2 domain-containing protein B1

- The rs7498665 SNP in *SH2B1* gene, an enhancer of leptin and insulin signaling, has been linked to snacking behavior but not to AN risk. There are no studies in other ED.

Executive summary (cont.)

Mitochondrial carrier homolog 2

- Rs3817334 is positively associated with emotional eating. In addition, carriers of the rs10838738 SNP showed a preference for the intake of saturated fat and carbohydrates.

Other genes near obesity loci identified by GWAS

- SNPs in or near *KCTD15*, *TMEM18*, *GNPDA2* and *ETV5* have been associated with BMI. However, only one study, which exclusively included AN patients, has assessed their role in ED with inconclusive results.

Perspective & conclusion

- There is an evident need for more studies in ED patients, especially BN and BED, that examine genes that are expressed in the hypothalamus and are involved in the molecular mechanisms of control of food intake and body weight.
- Recent evidence supports the notion that caution should be exerted when extrapolating findings from GWAS in ED.
- Increasing the size, but especially the homogeneity, of association studies in ED is crucial to reach meaningful clinical conclusions. This is particularly important when dealing with quantitative variables such as the scores in scales measuring patients' personality dimensions.

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