Serotonin transporter gene polymorphism in eating disorders: Data from a new biobank and META-analysis of previous studies

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ABSTRACT
Objectives Growing interest focuses on the association between 5-HTTLPR polymorphism and eating disorders (ED), but published findings have been conflicting. Methods The Italian BIO.VE.D.A. biobank provided 976 samples (735 ED patients and 241 controls) for genotyping. We conducted a literature search of studies published up to 1 April 2015, including studies reporting on 5HTTLPR genotype and allele frequencies in obesity and/or ED. We ran a meta-analysis, including data from BIO.VE.D.A. – comparing low and high-functioning genotype and allele frequencies in ED vs. controls. Results Data from 21 studies, plus BIO.VE.D.A., were extracted providing information from 3,736 patients and 2,707 controls. Neither low- nor high-functioning genotype frequencies in ED patients, with both bi- and tri-allelic models, differed from controls. Furthermore, neither low- nor high-functioning allele frequencies in ED or in BN, in both bi- and triallelic models, differed from control groups. After sensitivity analysis, results were the same in AN vs. controls. Results remained unaltered when investigating recessive and dominant models. Conclusions 5HTTLPR does not seem to be associated with ED in general, or with AN or BN in particular. Future studies in ED should explore the role of ethnicity and psychiatric comorbidity as a possible source of bias.

Introduction
Eating disorders (ED) are severe, often chronic psychiatric conditions that are associated with high rates of organic complications (Oflay et al. 2013), psychiatric comorbidities (Rojo-Moreno et al. 2015), self-injurious behaviours (Favaro et al. 2008), and death (Zervas et al. 2015). Being multifactorial diseases, ED are thought to be caused by environmental, perinatal, psychological, cultural, physical, familiar and genetic factors. Other psychiatric diseases, such as depressive disorders and anxiety disorders, have been associated with genetic polymorphisms of the serotonin transporter promoter region (Shinozaki et al. 2013; Mushtaq et al. 2014; Talati et al. 2015; Watanabe et al. 2015). Also in ED, particular attention has been paid to a specific serotonin transporter gene-linked polymorphic region (5HTTLPR) polymorphism, a 5’ regulatory promoter region that has been modelled as being bi-allelic in nature depending on a 44-bp deletion or insertion, with the S allele being low-functioning, and the L allele being high functioning (Heils et al. 1996). Moreover, a tri-allelic model has been proposed, with the Lg (an L allele with G replacing A in its SNP sequence) functioning equivalent to the S allele (Hu et al. 2006; Zalsman et al. 2006). The fact that specific attention is focussed on the SHTTLPR polymorphism is due to the role of serotonin in appetite regulation and the fact that one of the evidence-based treatments suggested in guidelines for bulimia nervosa (BN) is a selective serotonin reuptake inhibitor (fluoxetine) (Aigner et al. 2011). Several studies have investigated the relationship between AN, BN and ED and SHTTLPR (Hinney et al. 1997; Di Bella et al. 2000; Fumeron et al. 2001; Lauzurica et al. 2003; Matsushita et al. 2004; Urwin et al. 2005; Frieling et al. 2006; Monteleone et al. 2006a, 2006b,...
Rybakowski et al. 2006; Ribases et al. 2008; Richardson et al. 2008; Steiger et al. 2008a, 2008b, 2009, 2011; Martášková et al. 2009; Sundaramurthy et al. 2000; Ehrlich et al. 2010; Karwautz et al. 2011; Castellini et al. 2012; Thaler et al. 2013). However, only one focused on binge ED (Monteleone 2006b), and only two compared patients affected by AN with an obese sample (Hinney et al. 1997; Fumeron et al. 2001). These studies have partially been meta-analysed with literature searches conducted up to July 2008 (Lee and Lin 2010) and October 2009 (Calati et al. 2011). However, more individual studies have explored this area since then (Martaskova et al. 2009; Ehrlich et al. 2010; Karwautz et al. 2011; Steiger et al. 2011; Castellini et al. 2012; Thaler et al. 2013).

Aims of the study

In this study, we aimed to summarise all published evidence about this important topic, and to contribute our own data from the BIO.VE.D.A biobank, providing 5HTTLPR frequencies from the largest sample of patients affected by ED investigated in comparison with healthy controls (HC) to date.

Methods

‘Biobanca veneta per i disturbi alimentari’ (BIO.VE.D.A.) data

The ‘Biobanca Veneta per i Disturbi Alimentari’ (BIO.VE.D.A.) sample included 735 patients with a lifetime history of AN and/or BN according to DSM-IV criteria, and 241 HC. Patients were recruited in five Eating Disorder Units of the Veneto region, Italy. The BIO.VE.D.A. project is funded by the Veneto Region and aims to create a genetic biobank for ED (Boraska et al. 2014). Inclusion criteria were a life-time history of AN and/or BN according to DSM-IV, (1) age >14 years old, patients’ and parental (if less than 18 years old) informed consent. The study was approved by the local hospital Ethic Committee. Exclusion criteria were organic comorbidity or major psychiatric comorbidity (bipolar disorder, schizophrenia, major depressive disorder). After informed consent, all participants underwent a saliva or blood sample. Genomic DNA was extracted from 200 μl of whole peripheral blood, using a High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH), or from 500 μl of saliva using an Oragene●DNA/saliva Kit (DNA Genotek Inc.) according to the manufacturer’s instructions.

The 5-HTT promoter region was amplified from genomic DNA to discriminate between the L- and S-alleles (rs4795541). Primer sequences were designed using Primer 3 software (http://frodo.wi.mit.edu/) (forward: CAACTCCCTGTACCCTCCT and reverse: GTGCAA GGAGAATGCTGGAG). This reaction produced a fragment of 297 bp for the long L allele and a fragment of 254 bp for the short S allele. A total of 200 ng of genomic DNA was amplified using 12.5 μl of pre aliquoted ReddyMix™ PCR Master Mix (Thermo Fisher Scientific, Milan, Italy), 10 × DMSO and 0.4 μM of each primer, in a total volume of 25 μl. Cycling conditions were: 1 cycle at 94°C for 4 min, followed by 37 cycles at 94°C for 1 min, at 61°C for 1 min and at 72°C for 1 min.

To genotype the rs25531 the amplification was followed by a restriction digestion. A total of 7 μl of PCR product was digested at 37°C for 2 h with 5 U of MspI (New England BioLabs, Celbio, Milan, Italy), which recognises the sequence 5′-CGG-3′. Fragments were separated on acrylamide gel at 10% (45 min at 200V). The LA allele produces fragments of 257 and 39 bp, while the Lg allele produces fragments of 174, 84 and 39 bp.

Meta-analysis

Search strategy

We conducted an electronic literature search in PubMed from database inception until 1 April 2015 for studies investigating 5HTTLPR biallelic or triallelic genotype or alleles frequencies in ED, with or without a control group. Controlled vocabulary terms (MeSH) and the following keywords were used in the search strategy: (“Anorexia Nervosa”[Mesh]) OR (“Bulimia Nervosa”[Mesh]) OR (“Eating Disorders”[Mesh]) OR (“Binge-Eating Disorder”[Mesh]) AND (serotonin transporter OR 5HTTLPR OR 5-HTTLPR)). Reference lists of included articles and those relevant to the topic were hand-searched for identification of additional potentially relevant articles.

Study selection

Included were only studies that: (1) included patients affected by ED according to DSM-IV criteria and (2) reported 5HTTLPR genotype or allele frequencies in patients affected by ED, and a control group, if present. Both biallelic and triallelic modelled studies were analysed. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) criteria (Von Elm et al. 2007) were used for the quality assessment of included studies. Previous reviews and meta-analyses were full-text read to identify further studies.

Data extraction and statistical analysis

Two authors (MS and DG) independently extracted data from the selected studies into a standardised Microsoft
Excel spreadsheet. Any disagreement was resolved by consensus. The following information was extracted: (i) study population characteristics (e.g. sample size, demographics, diagnostic criteria and subtype of AN or BN, comorbidities, medications, anthropometric data); (ii) biallelic or triallelic genotype or alleles frequencies in patients and controls; (iii) quality indicators used for the STROBE assessment; and (iv) Hardy–Weinberg equilibrium (HWE). Whenever studies had the same authors or affiliation or close publication year, we contacted authors to verify data were not referring to the same sample. Whenever genotype or allele frequencies were not available, we contacted authors to ask for unpublished data. Furthermore, we extracted data from a previous meta-analysis (Gorwood et al. 2004), reporting data not available in original papers. We ran the following comparisons within patients and within controls: (1) triallelic low-functioning genotype frequency (containing S or Lg) vs. high-functioning genotype (LaLa); (2) biallelic low-functioning genotype (SS or SL) vs. high-functioning (LL); (3) triallelic low-functioning allele frequency (S and Lg) vs. high-functioning allele frequency (La); (4) biallelic low-functioning allele frequency (S) vs. high-functioning allele frequency (L). Additionally, comparing ED, AN and BN patients with the respective healthy control group, we ran the following comparisons: (5) biallelic low-functioning genotype frequencies (SS or SL), including recessive model testing for the low-functioning allele; (6) biallelic low-functioning allele frequencies (S); (7) triallelic low-functioning genotype (containing S or Lg); and (8) triallelic low-functioning allelic frequencies (S and Lg). When an obese sample was used as a control group (Hinney et al. 1997; Fumeron et al. 2001), we compared it separately to the ED samples. Moreover, we tested also dominant models for the low-functioning allele in ED, AN and BN vs. HC. Finally we compared the bi-allelic model vs. triallelic model, reporting differences in frequencies from studies providing both bi-allelic and triallelic data. Grouping of SS and SL was based on the presumed dominant role of the S allele of 5HTTLPR polymorphism (Lesch et al. 1996). We meta-analysed comparisons reported in at least two studies, in order to provide the maximum number of meta-analyzable outcomes.

The meta-analysis was performed using Review Manager (RevMan) Version 5.1 for Windows (http://tech.cochrane.org/revman). When combining studies, the random effects model (Der Simonian and Laird 1986) was used to account for study heterogeneity. For dichotomous data, odds ratio (OR) with its 95% confidence interval (CI) was used. Study heterogeneity was measured using the chi-squared and I-squared statistics, with chi-squared P < 0.05 and I-squared ≥50% indicating significant heterogeneity (Higgins et al. 2003). If I-squared ≥50% a sensitivity test was performed, where each study, one at a time, was excluded from the overall OR calculation to examine if any single study contributed significantly to the overall result. Furthermore, since there is evidence of different distributions of 5HTTLPR in specific ethnicities, e.g. higher S allele frequencies in Japanese populations compared to Caucasian populations (Lesch et al. 1996; Ng et al. 2006), we ran a sensitivity analysis with studies including other than Caucasian populations. Finally, funnel plots were inspected visually to assess the possibility of a publication bias. We included Bio.Ve.D.A. group data in the meta-analysis. HWE was tested in each study separately with both chi-squared test and relative excess heterozygosity test (REH; Ziegler et al. 2011). Finally, we tested the pooled REH from all included studies using a random effect model, which is a more appropriate tool than the chi-squared test to test HWE in meta-analyses (Ziegler et al. 2011).

Results

Included studies

We included BIO.VE.D.A. data and data from 21 studies, according to the flow chart shown in Figure 1. We excluded one study (44) because it allowed for subclinical participants; however, this study was included in a previous meta-analysis (Calati et al. 2011). The main features of the 22 included studies are summarised in Table 1. Altogether, 16 studies compared patients affected by ED with non-affected controls; six studies did not include a control group. Of the 16 control groups, 12 studies used HCs, two included obese subjects (Hinney et al. 1997, Fumeron et al. 2001), one underweight subjects (Hinney et al. 1997), one normal eaters (Steiger et al. 2009), one healthy sisters (Karwautz et al. 2011), and one parents (Urwin et al. 2005). All studies defined ED according to DSM-IV criteria. Sixteen of the 22 studies used a biallelic model, six also used a triallelic (BIO.V.E.D.A.; Steiger et al. 2008a, 2008b, 2009, 2011; Thaler et al. 2013). The quality of the included studies is reported in Supplementary Table I (available online).

Analyses within patients and within controls

Results of the meta-analyses within patients and within controls are reported in Table 2. Within patients and within controls, low-functioning genotypes were more frequent than high-functioning ones (ED vs. controls, P < 0.0001).
Within patients and within controls, high-functioning alleles were more frequent than low-functioning ones in the biallelic model. This result held true even in a leave-one-out sensitivity analysis, which based on funnel plot inspection excluded Matsushita et al. (2004) from the analysis. However, adopting the triallelic model, no significant difference emerged between low- and high-functioning allele frequencies.

Comparing the two models, only including studies using both the biallelic compared with the triallelic model included 9.9% more low-functioning genotype and 13.7% more low-functioning alleles.

Analyses comparing patients vs. controls

All results of the meta-analyses comparing patients with control groups are reported in Table 3. Low-functioning genotype frequencies in patients groups, with both bi- and tri-allelic models, did not differ from control groups (Figure 2). Furthermore, in both bi- and triallelic models, low-functioning allele frequencies in ED and in BN patients (biallelic only), were not different from control groups. Low-functioning allele frequencies in AN, in the biallelic model, were significantly higher than in control groups ($P = 0.03$). However, we removed one clearly outlying study (Matsushita et al. 2004) from the analysis based on funnel plot inspection, and as the patient group was more than 3 times smaller than the control group; the result became non-significant ($P = 0.09$) (Figure 3). Finally, we did not find that a dominant model significantly explained the group difference between AN ($P = 0.13$, after removing Matsushita et al. 2004), BN ($P = 0.85$), or ED ($P = 0.37$) vs. HC.

**ED patients vs. obese patients**

Only two studies compared patients affected by ED with patients affected by obesity (Hinney et al. 1997; Fumeron et al. 2001). Low-functioning genotype and allele frequencies were not significantly different between the two groups (genotype $P = 0.37$; allele $P = 0.40$).

**BIO.VE.D.A. data**

The BIO.VE.D.A. sample includes 735 patients affected by ED and 241 HC. Among ED, 526 were affected by AN
| Study/country | AN/BN/control group diagnostic criteria | Control group (Y/N/H/C/other disease) | N | AN/BN | Age AN/BN | Age control | BMI AN/BN (kg/m²) | BMI control (kg/m²) | Onset age (years) | Measurement method/ Comorbidity Medications | Personality or symptom scale or other clinical data | Genotype in Hardy–Weinberg equilibrium |
|---------------|----------------------------------------|----------------------------------------|---|--------|------------|-------------|------------------|------------------|-----------------|--------------------------------------------|-------------------------------|-----------------------------------|
| **With control group** |
| **Hinney et al.** (1997) Germany | CIDI, DSM IV | Underweight students; obese cohorts combined | AN 96 | Obese 385 | F: 16.6 ± 3.4; M: 15.3 ± 0.9 | Under-weight: F:24.7 ± 3.9; M:26.1 ± 4.1 Obese: F 29.57 ± 6.63; M 29.73 ± 5.3 | BMI F:14.5 ± 1.5; M:13.9 ± 2.0 | Underweight: F:17.6 ± 0.8; M:19.0 ± 1.0 | OBESE F 30.5 ± 5.3; M 33.6 ± 4.27 | PCR, biallelic | Three factor eating questionnaire | Yes |
| **Di Bella et al.** (2000) Italy | DSM IV | HC | AN 56 (19 R e 37 BP) BN 50 | 120 | 112 | 16.6 ± 3.4; M:15.3 ± 0.9 | Under-weight: F:24.7 ± 3.9; M:26.1 ± 4.1 Obese: F 29.57 ± 6.63; M 29.73 ± 5.3 | BMI F:14.5 ± 1.5; M:13.9 ± 2.0 | Underweight: F:17.6 ± 0.8; M:19.0 ± 1.0 | OBESE F 30.5 ± 5.3; M 33.6 ± 4.27 | PCR, biallelic | – | Yes |
| **Fumeron et al.** (2001) France, Caucasians | DSM IV | HC overweight subjects | 65F 2M; AN | AN 138 | 18.1 | 13.6 ± 1.9 | 13.73 (minimum average BMI) | 22.02 | PCR, biallelic | – | Yes |
| **Sundaramurthy et al.** (2000) UK, Caucasian | DSM IV | HC | AN 138 | 90 | 18.1 | 13.6 ± 1.9 | 13.73 (minimum average BMI) | 22.02 | PCR, biallelic | – | Yes |
| **Lausarica et al.** (2003) Spain | DSM-IV | HC | AN 102 (36 previous AN) | 107 | 31.1 ± 10.34 | BMI 21.4 ± 6, prev AN 205 ± 6 | BMI 22.5 ± 5, prev AN 21.09 ± 3 | 23 ± 3 | Blood, PCR, biallelic | No major psychiatric comorbidity | SCID-I, BITE, others | Yes |
| **Matsushita et al.** (2004) Japan | DSM IV | HC with EAT 26 score <20, or minimum BMI <17.5 | F. 77 AN; 118 BN; 14 EDNOS | 290F 25.8 ± 6.6 | 25.3 ± 8.0 | BMI >17.5 | – | – | Blood, PCR, biallelic | – | Yes |
| **Urwin et al.** (2005) Australia | DSM-IV | Mothers and fathers | AN 114 | 106 trios, 8 duos | 16.7 ± 3.46 | Min 14.6 ± 1.7 | 199 ± 1.97 before hospitalization | – | PCR, biallelic | – | Yes |
| **Monteleone et al.** (2006a) Italy | DSM IV | HC | F 77 BED | 61 F Caucasian | – | – | – | – | Blood PCR biallelic | – | SCID I e II | Yes |
| **Monteleone et al.** (2006b) Italy | DSM IV | HC | 125 F BN P | 94 HC F | 18-44 range | 18-41 range | 18.4 ± 1.4 | – | Blood, PCR, biallelic | – | SCID I, II, TO-R | Yes |
| **Rylkozko et al.** (2006) Poland | DSM IV | HC | 132 AN F | 93 HC F | 17.6 ± 2.9 | 20.9 ± 1.6 | 20.69 ± 1.85 | – | Blood, PCR, biallelic | – | TCI | Yes |
| **Markovskov et al.** (2009) Czech Republic | DSM-IV | AN BP | 65 | 25.39 ± 5.12 | 25.76 ± 5.12 | 14.65 ± 1.38 | 20.69 ± 1.85 | – | Blood, PCR, biallelic | – | Yes |
| **Steiger et al.** (2009) Canada, Caucasian | DSM-IV | Normal-eaters | 93 | 25.92 ± 7.05 | 24.43 ± 6.24 | 21.39 ± 3.89 | 22.00 ± 2.55 | – | Blood, PCR, biallelic | 77 ED, 1 control diagnosis, 77 ED, 1 control diagnosis. | EDE, DAEP, BI, CES-D, SCID-I, DB4 | Yes |
| **Ehrlich et al.** (2010) Germany | DSM-IV, ICD-10 | HC | AN-R 9, AN-8P 9, BN-9P 99, BN-EDNOS 47, AN-EDNOS 13. AN-58, rec AN 36.58 | AN 58, rec AN 36.58 | 18.4 ± 2.9 | recAN 195 ± 3.3 | recAN 21.6 ± 2 | – | Blood, PCR, biallelic. | No major psychiatric comorbidity. | No psychotropic medications. | Yes | SCL-90R, SIAB-EX, ED-2 |

(continued)
| Study/country | AN/BN/control group diagnostic criteria | Control group | Study/age | BMI AN/BN (kg/m²) | BMI control (kg/m²) | Onset age (years) | Measurement method/ Comorbidity Medications | Personality or symptom scale or other clinical data | Genotype in Hardy–Weinberg equilibrium |
|---------------|--------------------------------------|---------------|-----------|------------------|---------------------|------------------|---------------------------------------------|-------------------------------------------------|------------------------------------------|
| Karwautz et al. (2011) Austria, UK, Spain, Caucasians | DSM-IV Healthy sisters | AN-R 58, AN-BP 70 | 128 | 25.35 ± 8.1 | 25.9 ± 8.7 | 18.4 ± 2.3 | 22.6 ± 7.9 | 16.5 ± 4.2 | Blood, PCR, biallelic | – | Yes |
| Castellini et al. (2012) Italy, Caucasian | DSM-IV HC | AN-BP 65, AN-R 48, BN-BP 88 | 150 | 26.54 ± 7.55 AN, 28.37 ± 7.59 BN | 26.07 ± 20.74 | 16.56 ± 2.6 | – | – | Blood, PCR, biallelic | AN 54.9%, BN 36.7% | Antidepressants AN 37.2%, BN 29.5%; Anxiolytics AN 22.1%, BN 18.2% | – | Yes |
| BIOVE.D.A. group Italy, Caucasian | DSM-IV HC | ED 735 (AN lifetime 526, BN lifetime 341) | 241 | ED 25.56 (8.97); AN lifetime 25.01 (8.94); BN lifetime 26.45 (8.36) | 25.84 (5.71) | 16.58 (2.71) | 21.59 (2.93) | – | Blood and saliva, PCR, biallelic and triallelic | – | – | Yes |
| Sixteen studies DSM-IV 16/16 | AN 1679, BN 879; total 2749 with EDNOS and BED | AN 1679; BN 879; total 2707 | 23.8 ± 8 | 29.74 ± 11.12 yo | 18.79 ± 4.55 | 26.29 ± 6.79 | Reported in 2/16 studies | 14/16 biallelic only, reported in 5/16 studies | Reported in 3/16 studies | Reported in 9/16 studies | 15/16 |
| No control group | DSM-IV | N | ANR 2, ANP 1A, BNP 21, BNBP 3 | AN 26.83 ± 10.43; AN lifetime 25.84 ± 7.96 | AN 25.84 ± 7.96 | – | – | Blood, PCR, biallelic | SCID I, II, EDI2 | Yes |
| Ribas et al. (2000) Spain, Caucasian | DSM-IV N | AN 46 F; BN 36 F | – | AN 24.6 ± 4.4; BN 25.1 ± 6.35 | – | – | Anorexia Nervosa | – | SCID I e SCL 90-R | Yes |
| Steiger et al. (2008a) | DSM-IV | N | 65 BN P F; 4 BN NP 17 BN NAS F | 25.29 ± 6.4 | 22.39 ± 2.67 | – | – | Blood, PCR, biallelic and triallelic | Depression, anxiety, alcohol or drug abuse | – | – | Yes |

(continued)
| Study/country | AN/BN/control group diagnostic criteria | Control group (Y/N/HC/other disease) | N AN/BN | N Control group | Age AN/BN | Age control | BMI AN/BN (kg/m²) | BMI control (kg/m²) | Onset age (years) | Measurement method/ Comorbidity/Medications | Personality or symptom scale or other clinical data | Genotype in Hardy-Weinberg equilibrium |
|---------------|----------------------------------------|---------------------------------------|---------|----------------|-----------|-------------|------------------|-------------------|-----------------|---------------------------------------------|---------------------------------------------|-------------------------------------------|
| Steiger et al. (2008b) Canada, 95 white European; 2 Latin American; 1 mix asian | DSM IV | N | 72 BN R, 3 BN NP, 23 BN NOS | 26.81 ± 7.15 | – | 22.38 ± 3.90 | – | Blood, PCR, biallelic and triallelic | CES-D, BASIS-32, BIS-11 | Yes |
| Steiger et al. (2011) Canada, Germany, Caucasian | DSM-IV | AN-R 63, AN-BP 59, BN 221, EDNOS 56 | 25.04 ± 5.93 (<50) | – | 19.88 ± 4.32 (<35) | – | Blood, PCR, triallelic | EDE, SIAB-EX, SCID-II Self-harm | Yes |
| Thaler et al. (2013) Canada, 95.3% Caucasian | DSM-IV | N | 177 BN purge, 14 BN non purge; 82 EDNOS | 25.91 ± 6.62 | – | 22.62 ± 3.84 | – | Blood, PCR, SCID-II (hierarchical linear regression) | 127 on medications | EAT-26, DAPP, BIS, SCID-II |
| Six studies | DSM-IV 6/6 | N | AN 189 BN 660, EDNOS 138; total 987 | 25.51 ± 11.03 Yo N | N | 21.20 ± 4.20 N | Reported in 2/6 studies | 2/6 biallelic, 4/6 triallelic | Reported in 1/6 | Reported in 2/6 | Reported in 6/6 |
| OVERALL Total 22 studies | All DSM-IV | N | AN 1868; BN 1639; Total ED 3736 | 24.27 ± 8.9 yo | 29.74 ± 11.12 yo | 19.43 ± 4.58 | 26.29 ± 6.79 | Reported in 4/22 studies | 16/22 biallelic, 6/22 triallelic | 5/22 | 15/22 |

AN, anorexia nervosa; AN-BO, AN bulimic purgative; ANR, AN restricter; BASIS-32, Behaviour and Symptom Identification Scale; BIS, Barratt Impulsiveness Scale; BN, bulimia nervosa; CAPS, Clinical-Administered Post-Traumatic Stress Disorder Scale; CES-D, Centre for Epidemiological Studies Depression; CTI, childhood trauma interview; DAPP, dimensional assessment for personality pathology; DIS4, Diagnostic Interview Schedule, Version IV; EAT-26, Eating Attitude Test; EDE, eating disorder examination; EDNOS, eating disorder not otherwise specified; HC, healthy control; PCR, polymerase chain reaction; SCID-II, Structured Clinical Interview for DSM-IV Axis II diagnoses; SCL-90, Symptom Checklist 90 – Revised; SIAB-EX, Structured Interview for Anorexic and Bulimic Syndromes; TCI-R Temperament and Character Inventory Revised; yo, years old.
Table 2. High and low-functioning genotype and allele frequencies within patients and controls.

| Comparison | Number of studies | Studies | Participants or alleles | Statistical method | Effect estimate [95% CI]* |
|------------|-------------------|---------|-------------------------|--------------------|---------------------------|
| Biallelic genotype low vs. high ED | 21 | (BIO.VE.D.A. group; Castellini et al. 2012; Di Bella et al. 2000; Ehrlich et al. 2010; Frieling et al. 2006; Fumeron et al. 2001; Hinney et al. 1997; Karwautz et al. 2011; Lauzurica et al. 2003; Martaskova et al. 2009; Matsushita et al. 2004; Monteleone et al. 2006; Ribaes et al. 2008; Richardson et al. 2008; Steiger et al. 2008b; Rybakowski et al. 2006; Steiger et al. 2008a; Steiger et al. 2009; Sundaramurthy et al. 2000; Thaler et al. 2013; Urwin et al. 2005) | 3024 | Odds ratio (M-H, random. 95% CI) | 5.79 [4.10, 8.17] | P < 0.00001 |
| Triallelic genotype low vs. high ED | 6 | (BIO.VE.D.A. group; Rybakowski et al. 2006; Steiger et al. 2008a; Steiger et al. 2008b; Steiger et al. 2009; Thaler et al. 2013) | 1771 | Odds ratio (M-H, random. 95% CI) | 7.90 [6.50, 9.58]; P < 0.00001 |
| Biallelic vs. triallelic genotype ED | 5 | (BIO.VE.D.A. group; Richardson et al. 2008; Steiger et al. 2008b; Steiger et al. 2009; Thaler et al. 2013) | 1381 | Biallelic: 924 low 457 high. Triallelic: 1012 low 369 high |
| Biallelic genotype low vs. high controls | 16 | (BIO.VE.D.A. group; Castellini et al. 2012; Di Bella et al. 2000; Ehrlich et al. 2010; Fumeron et al. 2001; Hinney et al. 1997; Karwautz et al. 2011; Lauzurica et al. 2003; Martaskova et al. 2009; Matsushita et al. 2004; Monteleone et al. 2006; Ribaes et al. 2008; Richardson et al. 2008; Steiger et al. 2008b; Rybakowski et al. 2006; Steiger et al. 2008a; Steiger et al. 2009; Sundaramurthy et al. 2000; Thaler et al. 2013; Urwin et al. 2005) | 2146 | Odds ratio (M-H, random. 95% CI) | 4.95 [3.09, 7.92]; P < 0.00001 |
| Triallelic genotype low vs. high controls | 2 | (BIO.VE.D.A. group; Steiger et al. 2009) | 334 | Odds ratio (M-H, random. 95% CI) | 18.80 [3.81, 92.71]; P = 0.0003 |
| Biallelic vs. triallelic genotype controls | 2 | (BIO.VE.D.A. group; Steiger et al. 2009) | 334 | Biallelic: 233 low 101 high. Triallelic: 260 low 73 high |
| Biallelic alleles low vs. high ED | 14 | (BIO.VE.D.A. group; Castellini et al. 2012; Di Bella et al. 2000; Ehrlich et al. 2010; Fumeron et al. 2001; Hinney et al. 1997; Karwautz et al. 2011; Lauzurica et al. 2003; Martaskova et al. 2009; Matsushita et al. 2004; Monteleone et al. 2006; Ribaes et al. 2008; Richardson et al. 2008; Steiger et al. 2008b; Rybakowski et al. 2006; Steiger et al. 2008a; Steiger et al. 2009; Sundaramurthy et al. 2000; Thaler et al. 2013; Urwin et al. 2005) | 4566 | Odds Ratio (M-H, random, 95% CI) | 0.88 [0.55, 1.34]; P = 0.60; without Matsushita 0.69 [0.56, 0.85]; P = 0.0007 |
| Triallelic alleles low vs. high ED | 3 | (BIO.VE.D.A. group; Steiger et al. 2009, Steiger et al. 2011) | 2620 | Odds ratio (M-H, random. 95% CI) | 1.03 [0.79, 1.34]; P = 0.99 |
| Biallelic vs. triallelic alleles ED | 2 | (BIO.VE.D.A. group; Steiger et al. 2009) | 1840 | Biallelic: 785 low 1055 high. Triallelic: 890 low 940 high |
| Biallelic alleles low vs. high controls | 13 | (BIO.VE.D.A. group; Castellini et al. 2012; Di Bella et al. 2000; Fumeron et al. 2001; Hinney et al. 1997; Martaskova et al. 2009; Matsushita et al. 2004; Monteleone et al. 2006; Monteleone et al. 2006a; Monteleone et al. 2006b; Rybakowski et al. 2006; Steiger et al. 2009; Sundaramurthy et al. 2000; Thaler et al. 2013; Urwin et al. 2005) | 334 | Odds ratio (M-H, random. 95% CI) | 0.77 [0.42, 1.4]; P = 0.43; without Matsushita 0.62 [0.46, 0.85]; P = 0.003 |
| Triallelic alleles low vs. high controls | 2 | (BIO.VE.D.A. group; Steiger et al. 2009) | 666 | Odds ratio (M-H, random. 95% CI) | 1.28 [0.59, 2.77]; P = 0.54 |
| Biallelic vs. triallelic alleles controls | 2 | (BIO.VE.D.A. group; Steiger et al. 2009) | 666 | Biallelic: 296 low 372 high. Triallelic: 296 low 326 high |

*Effect estimate >1 indicates higher frequency of low-functioning genotype or alleles.

AN, anorexia nervosa; BN, bulimia nervosa; ED, eating disorder.
Table 3. Low-functioning genotype and allele frequencies in patients vs. controls.

| Comparison                  | Number of studies | Participants (patient vs. HC) or alleles | Statistical method | Effect estimate [95% CI]* |
|-----------------------------|-------------------|------------------------------------------|--------------------|--------------------------|
| **ED, AN, BN vs. controls** |                   |                                          |                    |                          |
| **Biallelic**               |                   |                                          |                    |                          |
| Low-functioning genotype SS | 16                | 2555 vs. 1936                           | Odds ratio (M-H, random, 95% CI) | 1.07 [0.87, 1.33]; P=0.51 |
| or SL (recessive model)     |                   |                                          |                    |                          |
| ED vs. controls             | 16                | (BIO.VE.D.A. group; Castellini et al. 2012; Di Bella et al. 2000; Ehrlich et al. 2010; Fumeron et al. 2001; Hinney et al. 1997; Karwautz et al. 2011; Lauzurica et al. 2003; Martaskova et al. 2009; Matsushita et al. 2004; Monteleone et al. 2006a; Monteleone et al. 2006b; Rybakowski et al. 2006; Steiger et al. 2009; Sundaramurthy et al. 2000; Urwin et al. 2005) |                      |                          |
| AN vs. controls (Figure 2). | 13                | 1637 vs. 1688                           | Odds ratio (M-H, random, 95% CI) | 1.13 [0.96, 1.32]; P=0.15 |
| BN vs. controls             | 6                 | 824 vs. 1002                            | Odds ratio (M-H, random, 95% CI) | 1.29 [0.78, 2.14]; P=0.32 |
| **Low-functioning alleles** |                   |                                          |                    |                          |
| ED vs. controls             | 13                | 4487 vs. 3326                           | Odds ratio (M-H, random, 95% CI) | 1.05 [0.88, 1.26]; P=0.56 |
| **Triallelic**              |                   |                                          |                    |                          |
| Low-functioning genotype SS | 2                 | 920 vs. 333                             | Odds ratio (M-H, random, 95% CI) | 0.20 [0.01, 3.62]; P=0.27 |
| or SL (dominant model)      |                   |                                          |                    |                          |
| ED vs. controls             | 16                | 2555 vs. 1936                           | Odds ratio (M-H, random, 95% CI) | 1.10 [0.89, 1.37]; P=0.37 |
| AN vs. controls             | 13                | 1637 vs. 1688                           | Odds ratio (M-H, random, 95% CI) | 1.27 [1.01, 1.60]; P=0.04; without Matsushita 1.19 [0.95, 1.50]; P=0.13 |
| BN vs. controls             | 6                 | 824 vs. 1002                            | Odds ratio (M-H, random, 95% CI) | 1.03 [0.73, 1.46]; P=0.85 |
| **Low-functioning alleles** |                   |                                          |                    |                          |
| ED vs. controls             | 13                | 4487 vs. 3326                           | Odds ratio (M-H, random, 95% CI) | 1.05 [0.88, 1.26]; P=0.56 |
| AN vs. controls             | 10                | 2783 vs. 2830                           | Odds Ratio (M-H, Random, 95% CI) | 1.19 [1.01, 1.39]; p = 0.03; Without Matsushita 1.15 [0.98, 1.3]; p = 0.09 |
| BN vs. controls             | 5                 | 1444 vs. 1790                           | Odds ratio (M-H, random, 95% CI) | 1.10 [0.76, 1.59]; P=0.62 |

AN: anorexia nervosa; BN: bulimia nervosa; ED: eating disorder.
Demographic features, genotype and allele frequencies are displayed in Table 4. No differences were found in both bi- and tri-allelic allele frequencies, or in genotype frequencies, between ED, AN, BN and HCs, or between different EDs.

**Hardy–Weinberg equilibrium and relative excess heterozygosity test**

All included studies were consistent with HWE and REH (Ziegler et al. 2011) according to random effects model; only one study (Di Bella et al. 2000) reported data from a sample with a lower-than-expected I/L frequency.

**Discussion**

Several lines of evidence show that an abnormal functional activity of the 5-HT system might affect satiety, anxiety and mood disorders (Shinozaki et al. 2013; Mushtaq et al. 2014; Talati et al. 2015; Watanabe et al. 2015). However, according to our data, 5HTTLPR polymorphism did not appear to have any direct additive effect on the risk of developing ED. Thus, although two previous meta-analyses (Lee and Lin 2010; Calati et al. 2011) reported significant associations between the low-functioning allele of 5-HTTLPR polymorphism and the risk of having AN, the newly accumulated evidence shows contrasting evidence about the role of this polymorphism in ED – including AN. Similarly to what has been observed in other psychiatric disorders (Shinozaki et al. 2013; Mushtaq et al. 2014; Watanabe et al. 2015; Talati et al. 2015), an additive effect of a single polymorphisms is not a valid model to explain the pathogenesis of ED. Therefore, studies exploring interactive effects are needed.

In the present study, a comparison between patients vs. controls was performed to re-test previously investigated associations with updated evidence. Both in the BIO.VE.D.A. data and in our meta-analysis, we found no significant difference in low-functioning genotype and allele frequencies between ED and controls.

Regarding BN, a previous meta-analysis (Thaler et al. 2013) pointed out that the relationship between the serotonin transporter gene polymorphism variance and BN was still controversial. In our meta-analysis, we added not only one more study (Castellini et al. 2012) to the pooled data, but we added the BIO.VE.D.A. data as well, including evidence from the largest sample published up to now. Our data confirm, like the previous findings (Lee and Lin 2010), the absence of any difference in the 5HTTLPR polymorphism distribution in ED vs. controls. This finding seems to rule out serotonin transporter polymorphism as a causal factor per se in the ED pathogenesis.

Another unique contribution of this meta-analysis is the comparison of allele and genotype frequencies between patients with ED and individuals affected by obesity. This comparison also showed no difference between the two groups, suggesting no relationship between BMI and 5HTTLPR polymorphism, as previously reported in a psychiatric sample (Shinozaki et al. 2013). However, we acknowledge that a meta-analysis including only two studies is very limited, so more studies are needed in this area. Furthermore, more studies should investigate the link between serotonin transporter polymorphism and BMI, in both healthy and

**Figure 2.** Biallelic low-functioning genotype frequency in anorexia nervosa vs. healthy controls.
psychiatrically ill subjects. We also tried to investigate samples affected by BED in our analysis, but to our knowledge only one study (Monteleone et al. 2006b) has investigated serotonin transporter polymorphism in that population, precluding any meta-analytic assessment.

The second part of our analysis was to evaluate the impact of the triallelic model in comparison to the biallelic model. Since the biallelic model classifies Lg alleles as high-functioning, which are actually low-functioning, a triallelic model is preferred since it better describes the “functional” distribution of the different alleles and genotypes. To date, only three studies (BIO.VE.D.A., Thaler, and Steiger) in six studies used a triallelic model (Steiger et al. 2008a, 2008b, 2009, 2011; Thaler et al. 2013).

Finally, our analyses within patients and within controls add to the available knowledge about the epidemiological distribution of the 5HTTLPR genotype, showing that low-functioning genotypes were significantly more frequent than high-functioning genotypes in both ED and controls. This finding could be due to the fact that low-functioning genotypes include two out of three (SS + SL vs. LL in biallelic model) and five out of six (SS + SLa + SLg + LgLg + LgLa vs. LaLa in triallelic model) possible combinations, respectively. However, other reasons could also play a role, including pleiotropic effects of the 5HTTLPR polymorphism and unknown advantages of a low-functioning 5HTTLPR genotype under certain conditions. More research in this area is needed to further clarify this finding and its potential implications.

The present study has both strengths and limitations that need to be taken into consideration when interpreting its results. First, the BIO.VE.D.A. biobank provides the largest sample of patients affected by ED (compared to a healthy control group) that has been investigated regarding the polymorphism of the serotonin transporter gene. Furthermore, BIO.VE.D.A. data agree with the results of the meta-analysis (considering the sensitivity analysis invalidating the association of the S allele and S or SL genotype with ED compared to controls). Moreover the BIO.VE.D.A. sample is free from major psychiatric comorbidities (bipolar disorder, schizophrenia, major depressive disorder), removing the possibility of potential biases due to other major psychiatric disorders. Table 1 presents data showing how previous studies often did not control for depressive disorders that are frequently encountered in patients with ED (Rojo-Moreno et al. 2015; Favaro et al. 2008). Thus, authors firmly promote multi-centric

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Table 4. BIO.VE.D.A. Group 5HTTLPR frequencies in subjects with eating disorders.

| Diagnosis         | Genotype | Allele Frequency | Age (SD) | M, F |
|-------------------|----------|------------------|----------|------|
|                    | La/La    | La/Lg            | Lg/La    | S/La | S/Lg | S/S | Total* |
| AN lifetime       | 140 (26.6%) | 29 (5.6%)      | 3 (0.5%) | 235 (44.8%) | 23 (4.3%) | 96 (18.2%) | 526 |
| BN lifetime       | 105 (30.8%) | 12 (3.5%)       | 1 (0.3%) | 140 (41.1%) | 22 (6.4%) | 61 (17.9%) | 341 |
| Healthy controls  | 61 (25.3%) | 16 (6.6%)       | 2 (0.8%) | 111 (46.1%) | 5 (2.1%) | 46 (19.1%) | 241 |
| Eating disorders  | 210 (28.6%) | 34 (4.7%)       | 3 (0.4%) | 322 (43.8%) | 37 (5%)  | 129 (17.5%) | 735 |

AN, anorexia nervosa; BN, bulimia nervosa; ED, eating disorder.

The total number of subjects with eating disorders is smaller than their sum of subjects with AN and BN, as life-time AN and life-time BN was the defining criterion for subgroup membership, resulting in a subgroup of patients with both life-time AN and life-time BN.

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Figure 3. Biallelic low-functioning allele frequency in anorexia nervosa vs. healthy controls.
collaborations involving national health services both within and among countries. Second, with seven additional studies (Steiger et al. 2008b, 2011; Martásková et al. 2009; Ehrlich et al. 2010; Karwautz et al. 2011; Castellini et al. 2012; Thaler et al. 2013) published in the last 6 years since the last meta-analysis (Lee and Lin 2010; Calati et al. 2011), this meta-analysis increased the sample size from 2,105 to 3,736 patients and from 2,032 to 2,707 controls. Third with our search we also explored the relationship with BED and obesity, pointing out the need for more studies in order to build some reliable evidence on the relationship between 5HTTLPR, BED, and obesity. Fourth, we stressed the need for more Asian studies, since compared to Caucasians the S allele seems more frequent in Asian people, in particular in the Japanese (Lesch et al. 1996; Ng et al. 2006). Fifth, compared to the meta-analysis by Lee and Lin (2010), we increased the sample size of BN from 395 to 824 patients, without mixing in sub-clinical samples (Wonderlich et al. 2005), as Calati et al. (2011) did. While we agree that a dimensional investigation is necessary to explain the relationship between symptoms and genes as proposed by Calati et al. (2011), we suggest that specifically designed studies are needed to provide consistent evidence on this dimensional aspect of ED. Conversely, mixing in minimally symptomatic subjects with ES is not helpful, adding more noise than clarifying dimensionality aspects. Moreover, our paper differs from Calati et al. (2011), in that we only meta-analysed studies with comparisons between patients and control groups, instead of building a virtual control group for studies without concurrently collected control groups.

The main limitations of the present study were: (1) the lack of studies controlling for comorbid anxiety, mood disorders, and suicidal behaviour, all possibly associated with 5HTTLPR polymorphism (Courtet et al. 2001; Lin et al. 2004); (2) few studies included Asian patients, precluding a better understanding of the relationship between 5HTTLPR polymorphisms and ED in that ethnic group; (3) there were frequent definitions of control groups as not being affected by ED, without clear exclusion of other organic or psychiatric diseases; (4) there was a lack of studies to date reporting data using the new diagnostic criteria using DSM-5 (APA, 2013); and (5) only two studies compared ED with obesity, which renders these results preliminary.

In conclusion, the present meta-analysis did not confirm results from previous single studies hypothesizing an additive major role of 5HTTLPR polymorphism for the risk of developing an ED. However, data provided by the present meta-analysis cannot rule out a possible small additive effect of 5-HTTLPR (that could be demonstrated only in very large samples), or an interactive effect. Thus, future studies should be designed to overcome the limitations of available studies, including a better definition of healthy control groups, the use of triallelic models, assessment of the effects of psychiatric comorbidity and ethnic differences, as well as providing data about possible environmental risk factors – such as stressful and traumatic events – that might interact with 5-HTTLPR polymorphism in increasing the risk for developing an ED.

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Statement of interest

None to declare.

References

Aigner M, Treasure J, Kaye W, Kasper S, WFSBP Task Force on Eating Disorders. 2011. World Federation of Societies of Biological Psychiatry (WFSBP) Guidelines for the pharmacological treatment of eating disorders. World J Biol Psychiatr. 12:400–443.

American Psychiatric Association. 2013. Diagnostic and statistical manual of mental disorders. (5th ed). Arlington, VA: American Psychiatric Publishing.

Courtet P, Baud P, Abbar M, Boulenger JP, Castelnau D, Mouthon D, Malafosse A, Buresi C. 2001. Association between violent suicidal behavior and the low activity allele of the serotonin transporter gene. Mol Psychiatr. 6:338–341.

Der Simonian R, Laird N. 1986. Meta-analysis in clinical trials. Contr Clin Trials. 7:177–188.

Di Bella DD, Catalano M, Cavallini MC, Riboldi C, Bellodi L. 2000. Serotonin transporter linked polymorphic region in anorexia nervosa and bulimia nervosa. Mol Psychiatr. 5:233–234.

Ehrlich S, Franke L, Scherag S, Burghardt R, Schott R, Schneider N, Brockhaus S, Hein J, Uebelhack R, Lehmkuhl U. 2010. The 5-HTTLPR polymorphism, platelet serotonin transporter activity and platelet serotonin content in overweight and weight-recovered females with anorexia nervosa. Eur Arch Psychiat Clin Neurosci. 260:483–490.

Favaro A, Santonastaso P, Monteleone P, Bellodi L, Mauri M, Rotondo A, Erzegovesi S, Maj M. 2008. Self-injurious behavior
and attempted suicide in purging bulimia nervosa: Associations with psychiatric comorbidity. J Affect Disord. 105:285–289.

Frieling H, Römer KD, Wilhelm J, Hillemacher T, Kornhuber J, de Zwaan M, Jacoby GE, Bleich S. 2006. Association of Catecholamine-O-Methyltransferase and 5-HTTLPR genotype with eating disorder-related behavior and attitudes in females with eating disorders. Psychiatr Genet. 16:205–208.

Fumeron F, Betoulle D, Aubert R, Herbet B, Siest G, Rigaud D. 2001. Association of a functional 5-HT transporter gene polymorphism with anorexia nervosa and food intake. Mol Psychiatr. 6:9–10.

Gorwood P. 2004. Eating disorders, serotonin transporter polymorphisms and potential treatment response. Am J Pharmacogenom. 4:9–17.

Heils A, Teufel A, Petri S, Stöber G, Riederer P, Bengel D, Lesch KP. 1996. Allelic variation of human serotonin transporter gene expression. J Neurochem. 66:2621–2624.

Higgins JPT, Thompson SG, Deeks JJ, Altman DG. 2003. Measuring inconsistency in meta-analyses. BMJ. 327:557–560.

Hinney A, Barth N, Ziegler A, von Prittwitz S, Hahnen A, Takeda A, Shirakawa O, Higuchi S. 2004. Serotonin transporter linked polymorphic region: Allele distributions in relationship to body weight and in anorexia nervosa. Life Sci. 61:295–303.

Hu XZ, Lipsky RH, Zhu G, Akhtar LA, Taubman J, Greenberg BD, Xu K, Arnold PD, Richter MA, Kennedy JL, et al. 2006. Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. Am J Hum Genet. 78:815–826.

Karwautz AF, Wagner G, Waldherr K, Nader IW, Fernández-Aranda F, Estivill X, Holliday J, Collier DA, Treasure J. 2011. Gene-environment interaction in anorexia nervosa: Relevance of non-shared environment and the serotonin transporter gene. Mol Psychiatr. 16: 590–592.

Lauzurica N, Hurtado A, Escartí A, Delgado M, Barrios V, Morandé G. 2003. Polymorphisms within the promoter and the intron 2 of the serotonin transporter gene in a population of bulimic patients. Neurosci Lett. 352:226–230.

Lee Y, Lin PY. 2010. Association between serotonin transporter gene polymorphism and eating disorders: A meta-analytic study. Int J Eat Disord. 43:498–504.

Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Müller CR, Hamer DH, Murphy DL. 1996. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science. 274:1527–1531.

Lin PY, Tsai G. 2004. Association between serotonin transporter gene promoter polymorphism and suicide: Results of a meta-analysis. Biol Psychiatr. 55:1023–1030.

Martášková D, Slachtová L, Kemlink D, Záhoráková D, Papezová H. 2009. Polymorphisms in serotonin-related genes in anorexia nervosa. The first study in Czech population and meta-analyses with previously performed studies. Folia Biol (Praha). 55:192–197.

Matsushita S, Suzuki K, Murayama M, Nishiguchi N, Hishimoto A, Takeda A, Shirakawa O, Higuchi S. 2004. Serotonin transporter regulatory region polymorphism is associated with anorexia nervosa. Am J Med Genet B Neuropsychiatr Genet. 128B:114–117.

Monteleone P, Santonastaso P, Mauri M, Bellodi L, Erzegovesi S, Fuschino A, Favarò A, Rotondo A, Castaldo E, Maj M. 2006a. Investigation of the serotonin transporter regulatory region polymorphism in bulimia nervosa: Relationships to harm avoidance, nutritional parameters, and psychiatric comorbidity. Psychosom Med. 68:99–103.

Monteleone P, Tortorella A, Castaldo E, Maj M. 2006b. Association of a functional serotonin transporter gene polymorphism with binge eating disorder. Am J Med Genet B Neuropsychiatr Genet. 141B:7–9.

Mushtaq R, Shoib S, Shah T, Mushtaq S. 2014. 5Hydroxytryptamine transporter (SHTT) gene promoter region polymorphism in anxiety and depressive disorders. Med J Islam Repub Iran. 28:127.

Ng Ch, Easteal S, Tan S, Schweitzer I, Ho BK, Aziz S. 2006. Serotonin transporter polymorphisms and clinical response to sertaline across ethnicities. Prog Neuropsychopharmacol Biol Psychiatr. 30:953–957.

Oflaz S, Yucel B, Oz F, Sahin D, Ozturk N, Yaci O, Polat N, Gurdal A, Cizgici AY, Dursun M, Oflaz H. 2013. Assessment of myocardial damage by cardiac MRI in patients with anorexia nervosa. Int J Eat Disord. 46:862–866.

Ribasés M, Fernández-Aranda F, Gratacós M, Mercader JM, Casasnovas C, Núñez A, Vallejo J, Estivill X. 2008. Contribution of the serotoninergic system to anxious and depressive traits that may be partially responsible for the phenotypical variability of bulimia nervosa. J Psychiatr Res. 42:50–57.

Richardson J, Steiger H, Schmitz N, Joober R, Bruce KR, Israel M, Gauvin L, Anestin AS, Dandurand C, Howard H, de Guzman R. 2008. Relevance of the 5-HTTLPR polymorphism and childhood abuse to increased psychiatric comorbidity in women with bulimia-spectrum disorders. J Clin Psychiatr. 69:981–990.

Rojo-Moreno L, Arribas P, Plumed J, Gimeno N, García-Blanco A, Váz-Leal F, Vila ML, Livianos L. 2015. Prevalence and comorbidity of eating disorders among a community sample of adolescents: 2-year follow-up. Psychiatry Res. 227:52–57.

Rybakowski F, Slopian A, Dmitrzak-Wegrz M, Czerski P, Rajewski A, Hauser J. 2006. The 5-HT2A -1438 A/G and 5-HTTLPR polymorphisms and personality dimensions in adolescent anorexia nervosa: Association study. Neuropsychobiology. 53:33–39.

Shinozaki G, Kumar Y, Rosen BH, Rundell JR, Mrazek DA, Kung S. 2013. ‘Diminished’ association between the serotonin transporter linked polymorphism (SHTTLP PR) and body mass index in a large psychiatric sample. J Affect Disord. 151:397–400.

Steiger H, Joober R, Gauvin L, Bruce KR, Richardson J, Israel M, Anestin AS, Groleau P. 2008b. Serotonin-system polymorphisms (5-HTTLPR and -1438G/A) and responses of patients with bulimic syndromes to multimodal treatments. J Clin Psychiatry. 69:1565–1571.

Steiger H, Richardson J, Joober R, Israel M, Bruce KR, Ng Ying Kin NM, Howard H, Anestin A, Dandurand C, Gauvin L. 2008a. Dissocial behavior, the SHTTLP PR polymorphism and maltreatment in women with bulimic syndromes. Am J Med Genet B Neuropsychiatr Genet. 147B:128–130.

Steiger H, Richardson J, Schmitz N, Joober R, Israel M, Bruce KR, Gauvin L, Dandurand C, Anestin A. 2009. Association of trait-defined, eating-disorder sub-phenotypes with (biallelic and triallelic) 5HTTLPR variations. J Psychiatr Res. 43:1086–1094.

Steiger H, Fichter M, Bruce KR, Joober R, Badawi G, Richardson J, Groleau P, Rams C, Israel M, Bondy B, et al. 2011. Molecular-genetic correlates of self-harming behaviors in eating-disordered women: Findings from a combined study. J Affect Disord. 131:271–280.
Canadian-German sample. Prog Neuropsychopharmacol Biol Psychiatr. 35:102–106.
Sundaramurthy D, Pieri LF, Gape H, Markham AF, Campbell DA. 2000. Analysis of the serotonin transporter gene linked polymorphism (5-HTTLPR) in anorexia nervosa. Am J Med Genet. 96:53–55.
Talati A, Guffanti G, Odgerel Z, Ionita-Laza I, Malm H, Sourander A, Brown AS, Wickramaratne PJ, Gingrich JA, Weissman MM. 2015. Genetic variants within the serotonin transporter associated with familial risk for major depression. Psychiatr Res. 228:170–173.
Thaler L, Groleau P, Joober R, Bruce KR, Israel M, Badawi G, Sycz L, Steiger H. 2013. Epistatic interaction between SHTTLPR and TPH2 polymorphisms predicts novelty seeking in women with bulimia nervosa spectrum disorders. Psychiatr Res. 208: 101–103.
Urwin RE, Nunn KP. 2005. Epistatic interaction between the monoamine oxidase A and serotonin transporter genes in anorexia nervosa. Eur J Hum Genet. 13:370–375.
Von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. 2007. The strengthing the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. PLoS Med. 4:e296
Watanabe SY, Iga J, Numata S, Umehara H, Nishi A, Kinoshita M, Inoshita M, Ohmori T. 2015. Polymorphism in the promoter of the gene for the serotonin transporter affects the age of onset of major depressive disorder in the Japanese population. J Affect Disord. 183:156–158.
Wonderlich SA, Crosby RD, Joiner T, Peterson CB, Bardone-Cone A, Klein M, Crow S, Mitchell JE, Le Grange D, Steiger H, et al. 2005. Personality subtyping and bulimia nervosa: Psychopathological and genetic correlates. Psychol Med. 35:649–657.
Zalsman G, Huang YY, Oquendo MA, Burke AK, Hu XZ, Brent DA, Ellis SP, Goldman D, Mann JJ. 2006. Association of a triallelic serotonin transporter gene promoter region (5-HTTLPR) polymorphism with stressful life events and severity of depression. Am J Psychiatr. 163:1588–1593.
Zerwas S, Larsen JT, Petersen L, Thornton LM, Mortensen PB, Bulik CM. 2015. The incidence of eating disorders in a Danish register study: Associations with suicide risk and mortality. J Psychiatr Res. 65:16–22.
Ziegler A, Van Steen K, Wellek S. 2011. Investigating Hardy-Weinberg equilibrium in case-control or cohort studies or meta-analysis. Breast Cancer Res Treat. 128: 197–201.