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No evidence for the association of the serotonin transporter gene 5-HTTLPR polymorphism with anorexia nervosa: a systematic review and meta-analysis

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Abstract

The link between anorexia nervosa and the 5-HTTLPR polymorphism of the serotonin transporter 5-HTT gene, characterized by short and long alleles, has been explored in many candidate gene studies.

Meta-analyses of early studies indicated an association, suggesting that the short allele was a risk factor for the disorder. However, recent meta-analyses incorporating additional studies have revealed inconsistencies in those findings.

To provide updated insights into this matter, we conducted a new systematic literature review of articles published between 1997 and 2024 investigating the association between anorexia nervosa and the 5-HTTLPR 5-HTT polymorphism. Following PRISMA guidelines, we selected, evaluated and meta-analyzed fifteen studies.

When all studies were aggregated, with 2,021 patients and 2,232 controls, an association was observed (short-short vs. short-long + long-long genotypes, Fixed Effect, Odds Ratio = 1.29, 95% Confidence Interval = 1.11 – 1.51; p = 0.001), and (short allele vs. long allele, Fixed Effect, Odds Ratio = 1.15, 95% Confidence Interval = 1.05 – 1.26, p = 0.003). However, further analyses revealed that this finding was primarily due to one study conducted in Italy and two studies conducted in East Asia, whereas all other studies indicated no association. Geographic subgroup analysis performed on European studies confirmed the absence of an association across the regions considered.

These findings further suggest that the 5-HTTLPR polymorphism does not significantly contribute to the genetic susceptibility of anorexia nervosa. A more comprehensive understanding of the multifactorial etiology of anorexia nervosa may be attained through genome wide association studies and epigenetic studies.

Keywords: anorexia nervosa; candidate gene studies; serotonin; serotonin transporter; 5-HTT; 5-HTTLPR
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Introduction

The aim of this study is to re-evaluate the association between the 5-HTTLPR polymorphism of the serotonin transporter gene and anorexia nervosa (AN) using an updated and rigorous meta-analysis.

Together with other eating disorders (EDs), such as bulimia nervosa (BN) and binge eating disorder (BED), AN is a potential life-threatening multidimensional syndrome (DSM-5-TR; American Psychiatric Association, APA, 2022) with unclear pathogenesis. A widely accepted bio-psycho-social model (Frank, 2016) proposes a multidimensional causality that includes psychological, family/developmental, socio-cultural factors (Herpertz-Dahlmann et al., 2011; Berge et al., 2014; Tambelli et al., 2015; Langdon-Daly and Serpell, 2017), an intergenerational transmission of psychopathology (Cimino et al., 2016; Cerniglia et al., 2019), and genetic components (Bulik et al., 1998; Klump et al., 2001; Ben-Dor et al., 2002; Rask-Andersen et al., 2010; Trace et al., 2013; Hübel et al., 2018; Paolacci et al., 2020).

AN has a lifetime prevalence ranging from 0.2% to 3% (Smink et al., 2012; Treasure et al., 2015; Mayhew et al., 2018), predominantly affecting females, and is the most extensively investigated ED. AN is associated, among other factors, with cognitive impairments (Stedal et al., 2021; Keeler et al., 2022), and is characterized by a motivation to avoid eating due to a morbid fear of gaining weight (Treasure et al., 2015).

Over the past thirty years, research into the genetics of AN has employed various approaches such as candidate gene studies, linkage analyses, transmission disequilibrium tests (TDT), genome-wide association studies (GWAS) and epigenetic studies. Among these methods, candidate gene studies, which aim to examine the association between specific genes and AN, emerged in the late 1990s and rapidly expanded, sustained by initial promising results. However, they soon yielded inconsistent results due to several limitations that impeded a comprehensive understanding of the potential roles of the analyzed polymorphisms (Sher, 2000; Monteleone and Maj, 2008). Firstly, AN lacks a clear biological definition. This suggests that its neurobiological etiology is unlikely to be influenced by single genes and is more likely to be multifactorial. Additionally, many of the studies suffer from small sample sizes, geographic variations in genotype and allele frequencies, and population stratifications.

The serotonergic system has been a focal point in many of these studies. In fact, it is involved in regulating neurochemical pathways associated with mood, food intake, and body weight (Wyler et al., 2017), among other functions, and it appears to have a role in the etiology of AN (Kaye et al., 2005). Within this system, significant attention has been devoted to the genes encoding for the 5-HT2A receptor, 5-HTR2A, and the presynaptic transporter, 5-HTT, or SLC6A4,

Regarding 5-HTR2A, the main body of research has investigated association of AN with its rs6311 polymorphism. In a recent meta-analysis, we have reported updated information on this issue, suggesting that an association with AN cannot be assumed globally but appears limited to a specific geographic region, namely Italy, and thus dependent on a particular genetic and/or environmental context (Bevilacqua et al., 2024).

For where SLC6A4 is concerned, this gene is located on chromosome 17q11.2 and comprises 14 exons spanning 40 kb. The encoded protein is composed of 630 amino acids with 12 transmembrane domains. The serotonin transporter plays a significant role in regulating brain serotonin levels, and has pharmacological relevance being the major neuronal target of selective serotonin reuptake inhibitor (SSRI) antidepressants (Owens et al., 1997). It has been hypothesized that SLC6A4 expression is involved in cortical development and acquisition of cognitive function, with special regard to emotion-regulating circuits (Jedema et al., 2010). One SLC6A4 polymorphism that has garnered significant attention in studies focused on major depression, anxiety, obsessive-compulsive disorder (Hu et al., 2006), is the 5-HTTLPR or rs4795541 polymorphism, a genetic variation present in the transcriptional control region of this gene resulting in the presence (long allele, L) or absence (short allele, L) of a 44 bp long fragment (Heils et al., 1996; Lesch et al., 1996; Nakamura et al., 2000). This polymorphism has a functional effect since the L allele is associated with higher expression of the transporter compared to the S allele, and it in turn influences the levels of central serotonergic neurotransmission (Greenberg et al., 1999; Hu et al., 2006).

Several studies have suggested an implication of this polymorphism in behavioral aspects and/or disorders including measures of neuroticism, a risk factor for anxiety and depression (Lesch et al., 1996; Munafo et al., 2009), mood disorders (Karg et al., 2010), obsessive-compulsive disorder (Mak et al., 2015), autism (Nuñez-Rios et al., 2020), post-traumatic stress disorder (PTSD)(Gressier et al., 2013), impulsive behavior (Di Nocera et al., 2017). However, a clear role for this genetic variation has not been conclusively reported in any of these conditions (Margoob and Mushtag, 2011; Bevilacqua, 2018)

Many studies have also investigated the possible association of this polymorphism with EDs, supported by the recognized role of the SSRI fluoxetine in their pharmacological treatment (Aigner et al., 2011).

The results of on AN studies have been synthesized in various meta-analyses, namely those conducted by Gorwood (2004), Lee and Lin (2010), Calati et al. (2011) and Solmi et al. (2016).

Gorwood (2004) analyzed four early studies (Hinney et al., 1997; Di Bella et al., 2000; Sundaramurthy et al., 2000; Fumeron et al., 2001) including 357 patients and 470 controls, and reported a small but significant association with AN (S allele vs. L allele, Odds Ratio (OR) = 1.38; 95% Confidence interval (CI) = 1.16; 1.72; p = 0.015) and low heterogeneity. Similarly, Lee and Lin (2010) described a significant overall association (S allele vs. L allele, OR = 1.41; 95% CI = 1.20; 1.66, p < 0.0001) and very low heterogeneity after incorporating three additional studies (Lauzurica et al., 2003; Matsushita et al., 2004; Rybakowski et al., 2006), with a total of 602 patients and 960 controls. However, they noted that an association was absent in five of the seven studies, except for one study involving Italian cohorts (Di Bella et al., 2000) and one study involving Japanese cohorts (Matsushita et al., 2004). Calati et al. (2011) included two additional studies (Urwin et al., 2003; Ribasés et al., 2008) in their meta-analysis, but excluded the study by Lauzurica et al. (2003), comprising a total of 726 patients and 1013 controls. Their results indicated a much less significant association between the polymorphism and AN (S

allele vs. L allele, OR = 1.35; 95% CI = 1.07; 1.71, p = 0.04) with high heterogeneity.

In contrast to the previous reports, Solmi et al. (2016) did not find evidence for an association in their meta-analysis, which involved a total of 1637 patients and 1688 controls. This analysis included previous studies with the exclusion of the Urwin et al. study (2003), and incorporated six additional studies (Urwin and Nunn, 2005; Martásková et al., 2009; Ehrlich et al., 2010; Karwautz et al., 2011; Castellini et al., 2012; Solmi et al., 2016).

It is important to acknowledge that existing meta-analyses have some weaknesses such as the inclusion of studies with incorrect diagnoses or genotype data obtained from TDTs rather than case-control studies, studies lacking control cohorts, and a lack of subgroup analyses.

To draw more solid conclusions regarding 5-HTTLPR candidate gene studies on AN and rigorously evaluate their outcomes, we conducted a new and updated systematic review and meta-analysis. Our approach, developed after reviewing all reports and previous meta-analyses, involved evaluating various discriminating criteria, such as quality assessment, geographic location, participants' age and gender, diagnostic criteria, and analysis of anorexia nervosa subtypes. Taking all these factors into account, we performed an analysis based on a geographic evaluation of effect sizes, accompanied by appropriate statistical correction for multiple testing. The studies included in our analysis span almost three decades, from 1997 to 2024.

Methods

Article search and selection

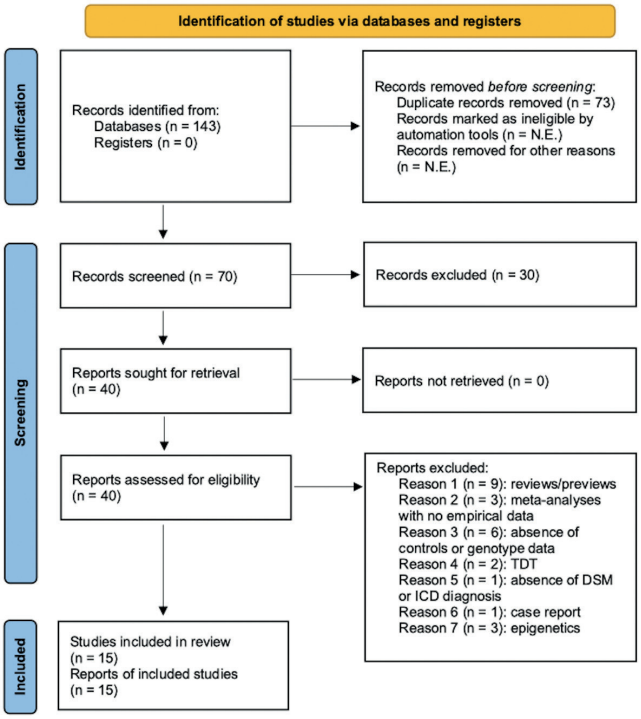
Publications were searched for in PubMed (https://pubmed.ncbi.nlm.nih.gov/, last accessed February 24th 2024) and EBSCO (https://search.ebscohost.com/, last accessed February 24th 2024) databases according to PRISMA guidelines (Page et al., 2021). Our searches covered the period between 1997, when the first candidate gene studies were published, and February 2024. In one search, using the terms "serotonin transporter" AND "anorexia nervosa" AND "human", we found the following numbers of publications: PubMed: 58, EBSCO: 50. In a second search, using the terms "5-HTTLPR" AND "anorexia nervosa" AND "human", we identified the following numbers of publications: PubMed: 16, EBSCO: 20. EBSCO databases included: MEDLINE, APA PsychInfo, CINAHL, Science Citation Index Expanded and Directory of Open Access Journals.

After merging the search results and removing duplicates, 70 papers were reviewed. Sixteen non-genetic papers, 13 papers not related to 5-HTTLPR or AN, and 1 paper involving animal models were excluded. The remaining 40 publications were selected based on the following criteria:

a) "study type" criterion: we included population-based, case-control genetic association studies and excluded patient-based studies and TDT studies;
b) "diagnosis" criterion: we included studies in which individuals with AN were diagnosed according to the DSM-IV (American Psychiatric Association, APA, 1994), DSM-5 (American Psychiatric Association, APA, 2013) or ICD-10 (World Health Organization, WHO, 1993), and control participants had no diagnosis of any pathology. We excluded studies in which participants' classification was performed by administered or self-administered questionnaires.

The search and selection of papers were performed independently by F. Santini and D. La Porta, reviewed and approved by all authors. Based on the above listed criteria, we excluded 8 reviews, 1 preview, 3 out of 4 meta-analyses that did not contain empirical data, 5 papers without control samples, 1 paper with no genotype data, 2 TDTs, 1 paper without DSM or ICD diagnosis, 1 case-report, and 3 epigenetic papers. Following the complete screening process, 14 research papers and 1 paper that included research and meta-analytical results, totaling 15 papers, were further analyzed (Figure 1). All papers were peer-reviewed and in English.

Fig. 1. PRISMA flow of study selection.



Meta-analysis

The selection, comparability and exposure qualities of the studies were assessed by the Newcastle–Ottawa quality rating scale (NOS) (Stang et al., 2010). Publication bias was evaluated by the Egger's test (Egger et al., 1997), the Begg's (Begg and Mazumdar, 1994) and funnel plots; Hardy–Weinberg (HW) equilibrium was assessed by the χ^2-test.

Data were collected independently by F. Santini and D. La Porta from the studies, they were reviewed and approved by all authors. Separate analyses for categorical variables were conducted to assess the association of each genetic polymorphism with AN. Genotype frequencies of individuals with AN and control participants from each study were analyzed using the following genetic models:

recessive model (SS vs. SL + LL), dominant model (SS + SL vs. LL), and allele model (S vs. L). Other models were not considered based on mathematical evidence indicating the effectiveness of these models in identifying the risk of single nucleotide polymorphisms in case-control genetic studies (Liu et al., 2021). To control for family-wise error rate, Bonferroni correction was applied, dividing the significance threshold by the number of genetic models tested ($p = 0.05/3$) (Pigott and Polanin, 2015). Thus, p values < 0.0166 were considered statistically significant.

Heterogeneity among the studies was assessed and quantified using a χ^2-based Q-test (Lau et al., 1997) and I^2 statistics (Higgins et al., 2002), respectively. The pooled OR and 95% CI was used for associations analyses, employing the Mantel-Haenszel fixed effects (FE) model (Mantel and Haenszel, 1959). The random effects model was not employed due to the low/moderate heterogeneity and variation observed across all results ($p > 0.1$ or $I^2 < 50.0\%$) (DerSimonian and Laird, 1986). The significance of the pooled ORs was determined using Z-tests. OR magnitudes were evaluated according to Cohen's criteria (Cohen, 1988), with the following thresholds: OR < 1.44, indicating a very small effect; 1.44 ≤ OR < 2.48, indicating a small effect; 2.48 ≤ OR < 4.27, indicating a medium effect; OR ³ ≥ 4.27, indicating a large effect.

To account for population stratifications, the geographic distribution of the studies was considered. Subgrouping criteria based on gender, age, DSM version, and NOS were not applied for the following reasons: most studies included only female participants, and some studies included a majority of female participants; age distribution, when disclosed, did not significantly differ across studies; the DSM-IV version was used across the studies; study quality, as indicated by NOS values, was largely consistent among the studies. Geographic subgroups were established only if they included at least three independent studies (Crocetti, 2016).

All analyses were conducted using Review Manager 5.3 (The Nordic Cochrane Centre, Copenhagen, DM) and MedCalc 20.211 (MedCalc Software Ltd, Ostend, Belgium).

Results

The present meta-analysis includes studies focused on the bi-allelic 5-HTTLPR or rs4795541 polymorphism of the 5-HTT or SLC6A4 gene (see Table 1).

Genotyping methods, diagnostic criteria, cohort compositions, and NOS evaluations of all included studies are reported in Table 2. Significance values of publication biases assessed by the Egger's test and Begg's test are presented in the legend of Figure 2. Funnel plots are reported in Figure 3.

The serotonin transporter gene, 5-HTT or SLC6A4, and its rs4795541 polymorphism, commonly referred to as 5-HTTLPR, have been extensively studied in various neuropsychiatric and behavioral contexts. This polymorphism, located in the promoter region of the gene, is characterized by two alleles, known as S and L. It has been investigated in several studies on AN (Hinney et al., 1997; Di Bella et al., 2000; Sundaramurthy et al., 2000; Fumeron et al., 2001; Lauzurica et al., 2003; Urwin et al., 2003; Matsushita et al., 2004; Urwin and Nunn, 2005; Rybakowski et al., 2006; Ribasés et al., 2008; Martásková et al., 2009; Ehrlich et al., 2010; Karwautz et al., 2011; Castellini et al., 2012; Gervasini et al., 2012; Chen et al., 2015; Solmi et al., 2016; Tenconi et

Tab. 1. Studies investigating the serotonin transporter gene rs4705541 (5-HTTLPR) polymorphism in the period 1997-2019, and inclusion/exclusion criteria of the present meta-analysis.

Author	Year	Gene	Polymorphism	Included/not included	Criterion for exclusion
Hinney et al.	1997	5-HTT	5-HTTLPR	Yes	
Di Bella et al.	2000	5-HTT	5-HTTLPR	Yes	
Sundaramurthy et al.	2000	5-HTT	5-HTTLPR	Yes	
Fumeron et al.	2001	5-HTT	5-HTTLPR	Yes	
Lauzurica et al.	2003	5-HTT	5-HTTLPR	No	Individuals with BN and previous AN episodes
Urwin et al.	2003	5-HTT	5-HTTLPR	No	TDT
Matsushita et al.	2004	5-HTT	5-HTTLPR	Yes	
Urwin and Nunn	2005	5-HTT	5-HTTLPR	No	TDT
Rybakowski et al.	2006	5-HTT	5-HTTLPR	Yes	
Ribasés et al.	2008	5-HTT	5-HTTLPR	No	no controls
Martásková et al.	2009	5-HTT	5-HTTLPR	Yes	
Ehrlich et al.	2010	5-HTT	5-HTTLPR	Yes	
Karwautz et al.	2011	5-HTT	5-HTTLPR	Yes	
Castellini et al.	2012	5-HTT	5-HTTLPR	Yes	
Gervasini et al.	2012	5-HTT	5-HTTLPR	Yes	
Chen et al.	2015	5-HTT	5-HTTLPR	Yes	
Collantoni et al.	2016	5-HTT	5-HTTLPR	Yes	
Solmi et al.	2016	5-HTT	5-HTTLPR	Yes	
Tenconi et al.	2016	5-HTT	5-HTTLPR	Yes	
Collantoni et al.	2019	5-HTT	5-HTTLPR	No	no genotype data

Note. AN, anorexia nervosa; BN, bulimia nervosa; TDT, transmission disequilibrium test.

Tab. 2. General characteristics of the studies concerning the 5-HTTLPR polymorphism included in the present meta-analysis.

Author	Year	Nation	Ethnicity	Genotyping method	Diagnostic criteria	Individual category	Male	Female	Total	Age	BMI	NOS
Hinney et al.	1997	Germany	Caucasian	PCR-LP	DSM-IV	Individuals with AN	4	92	96	M: 15.3±0.9 F: 16.6±3.4	M: 13.9±2.0 F: 14.5±1.5	8
						Healthy controls	64	48	112	M: 26.1±4.1 F: 24.7±3.9	M: 19.0±1.0 F: 17.6±0.8	
Di Bella et al.	2000	Italy	Caucasian	PCR-LP	DSM-IV	Individuals with AN	0	56	56	-	-	8
						Healthy controls	0	120	120	-	-	
Sundaramurthy et al.	2000	United Kingdom	Caucasian	PCR-LP	DSM-IV	Individuals with AN	0	138	138	18.1	13.73	8
						Healthy controls	0	90	90	30.28	22.02	
Fumeron et al.	2001	France	Caucasian	PCR-LP	DSM-IV	Individuals with AN	2	65	67	24.3±6.5	13.6±1.9	8
						Healthy controls	64	84	148	41.2±4.1	22.6±2.1	
Matsushita et al.	2004	Japan	Asian	PCR-LP	DSM-IV	Individuals with AN	0	77	77	25.8±6.6	-	8
						Healthy controls	0	290	290	25.3±8.0	-	
Rybakowski et al.	2006	Poland	Caucasian	PCR-LP	DSM-IV	Individuals with AN	0	132	132	17.6±2.9	-	8
						Healthy controls	0	93	93	20.9±1.6	-	
Martásková et al.	2009	Czechia	Caucasian	PCR-LP	DSM-IV and ICD-10	Individuals with AN	0	72	72	25.4±6.2	14.7±1.4	8
						Healthy controls	0	65	65	25.8±5.1	20.7±1.9	
Ehrlich et al.	2010	Germany	Caucasian	PCR-LP	DSM-IV	Individuals with AN	0	acAN: 57 recAN: 36	93	acAN: 17.4±2.7 recAN: 19.5±3.3	acAN: 14.2±1.4 recAN: 20.7±2.2	8
						Healthy controls	0	55	55	18.4±2.9	21.6±2.0	
Karwautz et al.	2011	Austria, U.K. Spain	Caucasian	PCR-LP	DSM-IV	Individuals with AN		116	116	16.5± 4.2	18.4±2.3	8
						Healthy controls		108	108	17.3±5.9	22.6 ±7.9	
Castellini et al.	2012	Italy	Caucasian	PCR-LP	DSM-IV	Individuals with AN	5	108	113	26.5±7.6	16.6±2.6	8
						Healthy controls	-	-	150	26.1±20.7	-	
Gervasini et al.	2012	Spain	Caucasian	PCR-LP	DSM-IV	Individuals with AN		67	67	-	-	8
						Healthy controls		222	222	-	-	
Chen et al.	2015	China	Asian	PCR-LP	DSM-IV	Individuals with AN	13	245	255	19.4±4.7	16.2±2.7	8
						Healthy controls	12	339	351	20.0±1.5	20.6±2.6	
Collantoni et al.	2016	Italy	Caucasian	PCR-LP	DSM-IV	Individuals with AN		acAN: 85 recAN: 47	132	23.5±7.3	acAN: 15.9 ±1.5 recAN: 21.0±2.1	8
						Healthy controls		106	106	23.7±6.6	21.5±3.2	
Solmi et al.	2016	Italy	Caucasian	PCR-LP	DSM-IV	Individuals with AN	-	-	526	25.01	17.47	7
						Healthy controls	-	-	241	25.84	21.59	
Tenconi et al.	2016	Italy	Caucasian	PCR-LP	DSM-IV and DSM-5	Individuals with AN	0	85	85	22.6±6.9	17.6±3.0	8
						Healthy controls	0	90	90	23.5±6.3	21.6±3.3	

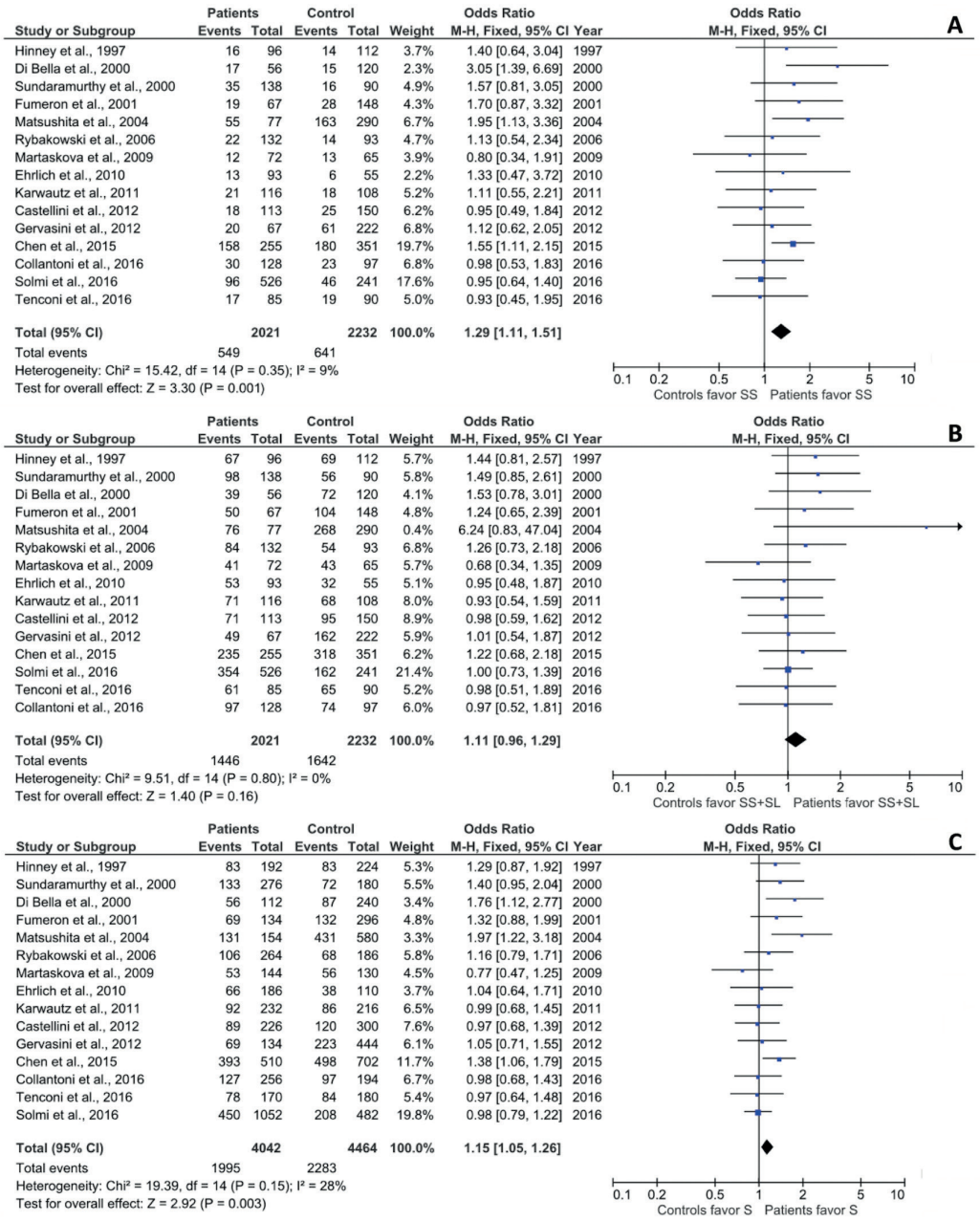
Note. Age = years ± S.D.; BMI= kg/m^2 ± S.D.; NOS: Newcastle-Ottawa Scale; DSM-IV: Diagnostic and Statistical Manual of Mental Disorders-IV (American Psychiatric Association, 1994); DSM-5: Diagnostic and Statistical Manual of Mental Disorders-5 (American Psychiatric Association, 2013); ICD-10: International Classification of Diseases-10 (World Health Organization, 1993); PCR-LP: polymerase chain reaction-length polymorphism; M: male; F: female; rAN: patients with restricting type AN; pAN: patients with purging type AN; acAN: patients with acute AN; recAN: patients with AN that had recovered.

al., 2016; Collantoni et al., 2016). In the present work, we excluded the Lauzurica et al. (2003) study, which was instead incorporated in the Lee and Lin (2010) meta-analysis, since its AN cohort consisted of individuals with BN who had experienced previous AN episodes. We also excluded the Urwin et al. (2003) study and the Ribasés et al. (2008) study, which were included in the Calati et al. (2011) meta-analysis. The former study was conducted using TDTs and the latter did not have controls. Additionally, we excluded the study by Urwin and Nunn (2005), which also relied on TDTs. For more details on included and excluded studies, refer to Table 1. The Gervasini et al. (2012) and the Tenconi et al. (2016) studies did not include complete genotype data, and we thank Drs. G. Gervasini and E. Tenconi for kindly providing the missing information upon request.

The studies included in present meta-analysis involved a total of 4,253 participants, comprising 2,021 patients and 2,232 controls. The NOS quality rating of the studies was 7.93 ± 0.26. Genotype and allele frequencies of patients and control samples were in HW equilibrium in eleven studies, except for the Chen et al. (2015) study, which involved a deviating AN cohort ($p < 0.05$). Among cohorts of Asian participants studied by Matsushita et al. (2004) and Chen et al. (2015), the S allele exhibited frequencies in the range of 70% to 80%. These frequencies are substantially different from those below or around 50% that were reported in all other studies. Similar allele frequencies in Asian populations have also been documented elsewhere (Haberstick et al., 2015).

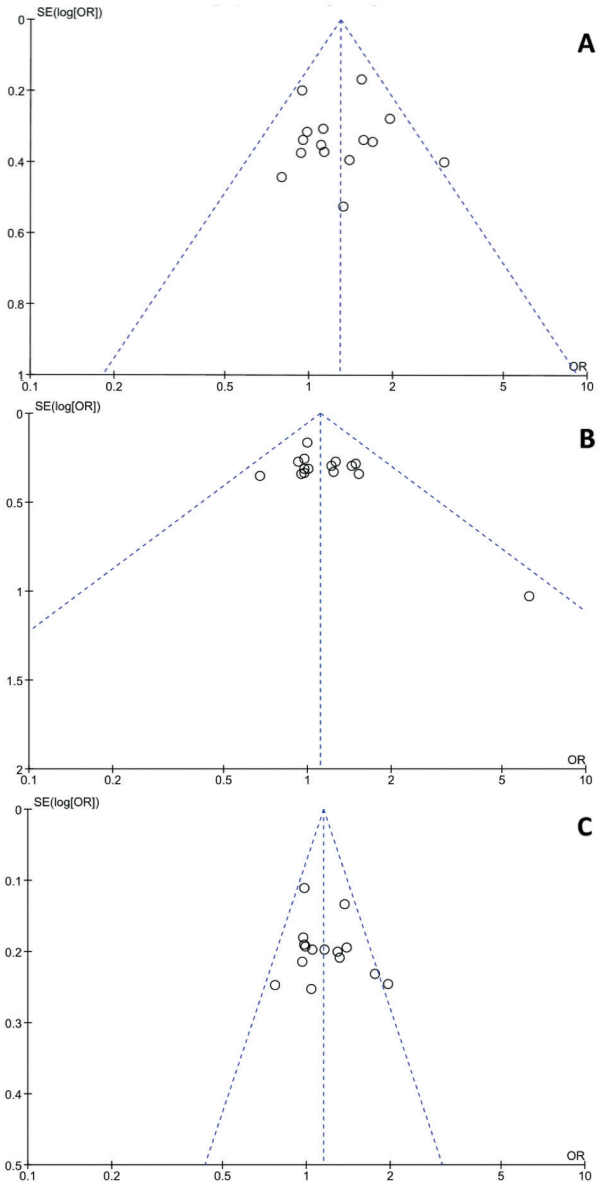
We first conducted global analyses using a fixed effects model, justified by the low/moderate values of heterogeneity and

Fig. 2. Odds ratios, 95% confidence intervals (CI), and forest plots of individual studies and relative pooled results between AN and the 5-HTTLPR polymorphism in different genetic models: A, recessive (SS vs. SL + LL); B, dominant (SS + SL vs. LL); and C, allele (S vs. L). Egger's test for publication bias: A, p = 0.8710; B, p = 0.1030; C, p = 0.4776. Begg's test for publication bias: A, p = 0.9605; B, p = 0.5200; C, p = 0.1815.



variation observed, and Bonferroni correction due to the three genetic models employed. A very small buy significant association with AN and the SS genotype was observed using the recessive model (FE, OR = 1.29, 95% C.I. = 1.11; 1.51; Z = 3.30, p = 0.001), with minimal heterogeneity and variation (χ^2 = 15.42; df = 14; p = 0.35; I^2 = 9%) (Figure 2A). Conversely, no association was observed using the dominant model (FE, OR = 1.11 95% CI = 0.96; 1.29, Z = 1.40, p = 0.16), with very limited heterogeneity and no variation (χ^2 = 9.51; df = 14; p = 0.80; I^2 = 0%) (Figure 2B). Lastly, the analysis of allele frequencies confirmed the presence of a very small association of the S allele with AN (FE, OR = 1.15, 95% CI = 1.05; 1.26; Z = 2.92; p = 0.003). Some effect size heterogeneity and result variation were observed (χ^2 = 19.39; df = 14; p = 0.15; I^2 = 28%) (Figure 2C). Analyses by a random effects model performed for a comparison, showed no significant differences (not shown). There was no publication bias in these analyses, as revealed by Egger's and Beggs's tests (legend to Figure 2), and funnel plots (Figure 3).

Fig. 3. Funnel plots of global studies on AN and the 5-HTTLPR polymorphism in different genetic models: A, recessive (SS vs. SL + LL); B, dominant (SS + SL vs. LL); and C, allele (S vs. L).



In summary, the analysis of all included studies reveals low heterogeneity among them, and consequent low clinical or methodological differences, and suggests that: i) the 5-HTTLPR S allele is prevalent among individuals with AN, and ii) its homozygous presence is associated with an increased risk of developing AN. Conversely, the L allele is more commonly found in healthy controls, and its presence appears to offer a protective effect, both in homozygous and heterozygous carriers.

To address population stratification, we divided the studies into distinct geographic regions based on the origins of all cohorts: Central Europe and Southern Europe. Other subgroups could not be formed due to the statistical criterion established. NOS quality ratings displayed no significant variations among these subgroups (data not shown). Below are the compositions of the two subgroups and their respective results.

The Central European subgroup included 460 patients and 473 controls from the following countries: Germany (Hinney et al., 1997; Ehrlich et al., 2010); France (Fumeron et al., 2001); Poland (Rybakowski et al., 2006); Czechia (Martásková et al., 2009). In this subgroup, no association was observed according to all models: a) recessive model, (FE, OR = 1.27, 95% CI = 0.89; 1.80; Z = 1.31; p = 0.19), with very low heterogeneity and no variation (χ^2 = 1.97; df = 4; p = 0.74; I^2 = 0%) (Figure 4A); b) dominant model, (FE, OR = 1.12, 95% CI = 0.85; 1.48; Z = 0.80, p = 0.42), with low heterogeneity and no variation (χ^2 = 3.26; df = 4; p = 0.52; I^2 = 0%) (Figure 4B); and c) allele model, (FE, OR = 1.13, 95% CI = 0.93; 1.37; Z = 1.26; p = 0.21), with low heterogeneity and no variation (χ^2 = 3.54; df = 4; p = 0.47; I^2 = 0%) (Figure 4C).

The Southern European subgroup comprised five Italian studies (Di Bella et al., 2000; Castellini et al., 2012; Solmi et al., 2016; Tenconi et al., 2016; Collantoni et al., 2016) and one Spanish study (Gervasini et al., 2012) with 975 patients and 920 controls. Similar to the previous subgroup, no association emerged in this subgroup according to the three models: a) recessive model, (FE, OR = 1.08, 95% CI = 0.86; 1.38; Z = 0.67, p = 0.50), with low heterogeneity but substantial variation (χ^2 = 7.55; df = 5; p = 0.18; I^2 = 34%) (Figure 5A); b) dominant model, (FE, OR = 1.04, 95% CI = 0.84; 1.27; Z = 0.33, p = 0.74), with almost no heterogeneity and no variation (χ^2 = 1.44; df = 5; p = 0.74; I^2 = 0%) (Figure 5B); and c) allele model, (FE, OR = 1.04, 95% CI = 0.91; 1.20; Z = 0.60; p = 0.55), with low heterogeneity but some variation (χ^2 = 5.70; df = 5; p = 0.34; I^2 = 12%) (Figure 5C).

We excluded from the subgroup analysis two European studies, which did not suggest an association, one involving British cohorts (Sundaramurthy et al., 2000), the other focused on pooled Austrian, British and Italian cohorts (Karwautz et al., 2011), and two Asian studies (Matsushita et al., 2004; Chen et al., 2015), which displayed an association according to the recessive model (data not shown).

In summary, unlike the observations from the global analysis, the 5-HTTLPR polymorphism did not demonstrate any association with AN in the two geographic subgroups, as determined by the distribution of the studies, according to all genetic models.

Fig. 4. Odds ratios, 95% confidence intervals (CI), and forest plots of individual Central European studies and relative pooled results between AN and the 5-HTTLPR polymorphism in different genetic models: A, recessive (SS vs. SL + LL); B, dominant (SS + SL vs. LL); and C, allele (S vs. L).

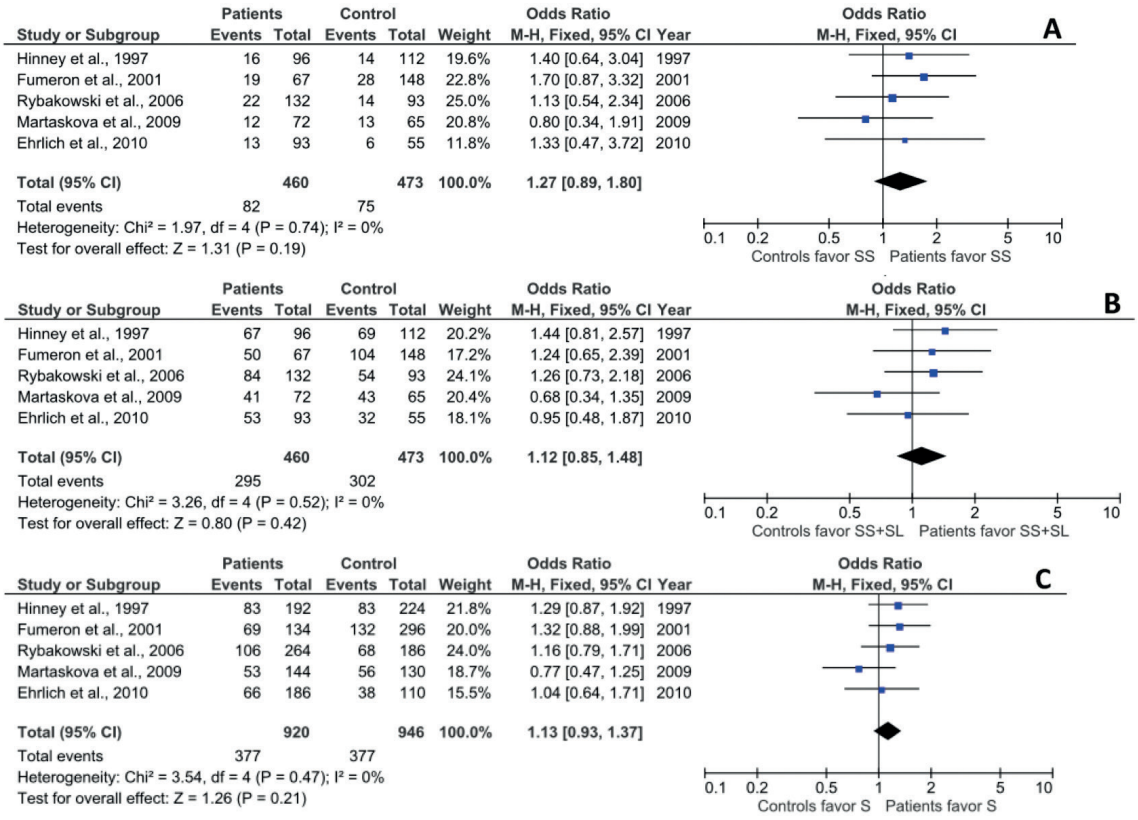
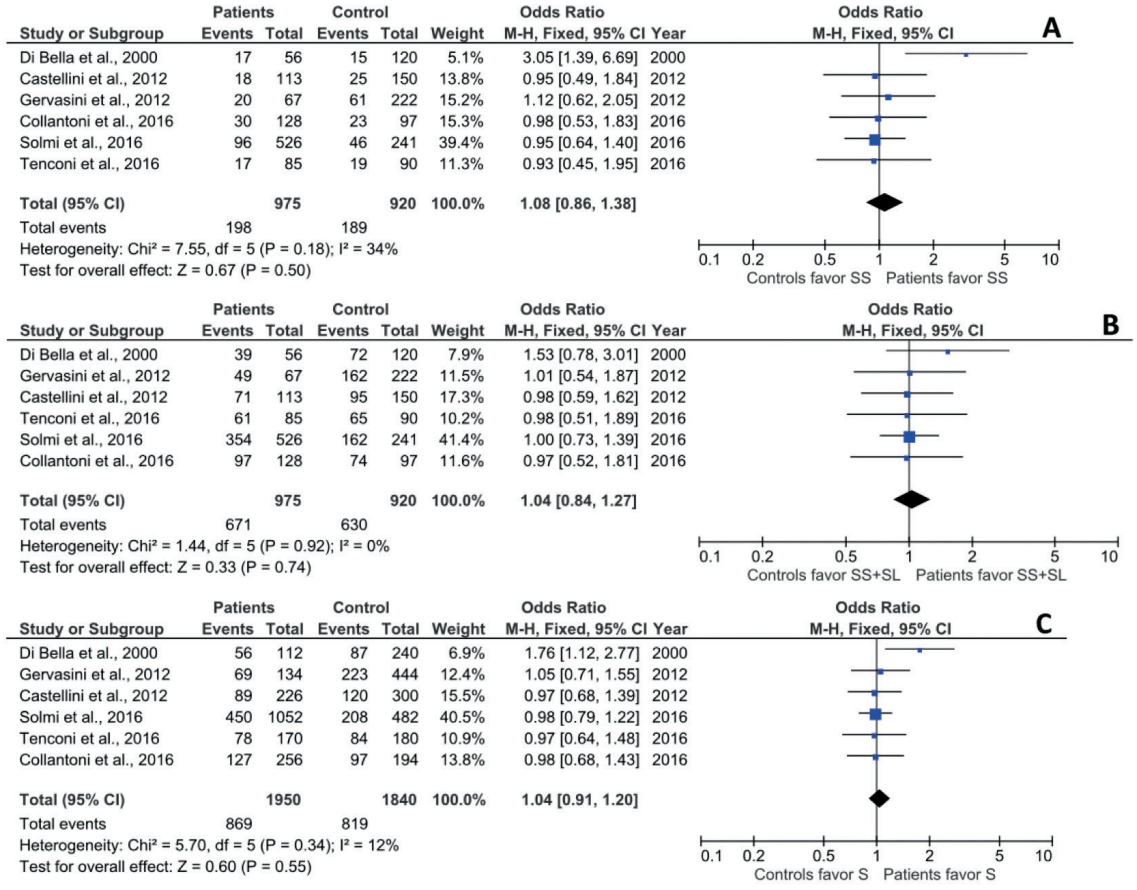


Fig. 5. Odds ratios, 95% confidence intervals (CI), and forest plots of individual Southern European studies and relative pooled results between AN and the 5-HTTLPR polymorphism in different genetic models: A, recessive (SS vs. SL + LL); B, dominant (SS + SL vs. LL); and C, allele (S vs. L).



Discussion

The 5-HTTLPR polymorphism of the 5-HTT gene, characterized by the presence of 14 (S allele) or 16 (L allele) non-identical repeat units, has been extensively investigated for its association with various behavioral aspects, neuropsychiatric conditions, and traits (Sharpley et al., 2014; Walitza et al., 2014; Gatt et al., 2015).

In vitro studies conducted on non-neuronal cells have shown that the L variant is transcribed up to three-fold more efficiently than the S variant, leading to higher transporter expression and serotonin reuptake (see Iurescia et al. (2016) for a review). This observation, coupled with the known effects of SSRI antidepressants, suggests that the L variant would represent a psychopathological risk factor. However, some studies have proposed that the presence of the S allele is associated with a heightened response of the hypothalamic-pituitary-adrenal (HPA) axis to psychosocial stress (Way and Taylor, 2010), and increased neurochemical and physiological responses to stressful tasks (Ohira et al., 2009; Fredericks et al., 2010). Additionally, the S variant has been implicated in conditions such as bipolar disorder (Rao et al., 2019), neuroticism (Ibarra et al., 2011) or violent suicide (Fanelli and Serretti, 2019).

While this polymorphism has been largely investigated for its potential association with AN, our updated meta-analysis, despite describing an association in a global assessment, does not confirm its presence according to the geographic subgroup analysis. Specifically, analysis of effect sizes shows that the association observed for the entire group stems from only three out of fifteen studies: one conducted on Italian individuals (Di Bella et al., 2000) and two on Asian individuals (Matsushita et al., 2004; Chen et al., 2015).

We therefore excluded these studies from the meta-analysis in leave-one-out tests. Due to the absence of association observed for the dominant model, this model was not further analyzed.

Exclusion of the Di Bella et al. (2000) study produced reduced levels of association according to both the recessive model, (FE, OR = 1.25, 95% CI = 1.07; 1.46; Z = 2.83, p = 0.005, χ^2 = 10.64; df = 13; p = 0.64; I^2 = 0%) and the allele model (FE, OR = 1.13, 95% CI = 1.03; 1.24; Z = 2.48; p = 0.01, χ^2 = 15.82; df = 13; p = 0.26; I^2 = 18%).

Exclusion of the Matsushita et al. (2004) study produced a more pronounced reduction in the recessive model, (FE, OR = 1.25, 95% CI = 1.06; 1.46; Z = 2.71, p = 0.007, χ^2 = 13.06; df = 13; p = 0.44; I^2 = 0%) and the allele model (FE, OR = 1.12, 95% CI = 1.02; 1.23; Z = 2.36; p = 0.02, χ^2 = 14.31; df = 13; p = 0.35; I^2 = 9%).

Exclusion of the Chen et al. (2015) study produced the most pronounced effect according to both the recessive model (FE, OR = 1.23, 95% CI = 1.04: 1.46; Z = 2.36, p = 0.02, χ^2 = 13.93; df = 13; p = 0.38; I^2 = 7%] and the allele model (FE, OR = 1.12, 95% CI = 1.01; 1.24; Z = 2.21; p = 0.03, χ^2 = 17.25; df = 13; p = 0.19; I^2 = 25%).

Taking this into account, the existence of a global association cannot be considered further.

These findings refute the conclusions of the meta-analyses conducted by Gorwood (2004), Lee and Lin (2010, and Calati et al., (2011). Gorwood (2004) considered the results of the earliest four studies published on this issue, and an overall very limited number of both individuals with AN and controls. Although performed in various geographic regions, all studies displayed a higher frequency of the 5-HTTLPR S allele in individual with AN. Among the studies, a significant association with AN was found in Di Bella et al. (2000) and a tendency to association with AN in Sundaramurthy et al. (2000). Altogether, this meta-analysis described presence of association with low heterogeneity.

With time, additional studies reported a reduced prevalence of the S allele among AN cohorts and the second meta-analysis conducted by Lee and Lin (2010) on a total of 602 patients and 960 controls in seven studies, although still describing an overall association of the S allele with AN and very low heterogeneity, acknowledged the presence of an association in only two studies (Di Bella et al., 2000; Matsushita et al., 2004). A third meta-analysis was conducted by Calati et al. (2011) on eight studies comprising 726 individuals with AN and 1013 controls. The authors reported a much less significant association of the S allele with AN but a high heterogeneity among the studies.

In contrast, our results confirm those of the meta-analysis conducted more recently by Solmi et al. (2016), based on a higher number of studies and participants coupled to stricter criteria. In essence, our meta-analysis included only studies with accurate AN diagnoses and case-control groups. For these reasons, as reported in Table 1, studies by Lauzurica et al. (2003), Urwin et al. (2003), Urwin and Nunn (2005) and Ribasés et al. (2008), which were included in previous) meta-analysis, were excluded. We also excluded a recent study by Collantoni et al. (2019) that did not report genotype data.

Our results are also in line with recent reviews on the genetics of AN (Baker et al., 2017; Abou Al Hassan et al., 2021).

Similar to present observations, numerous studies have reported inconsistent results regarding associations of the 5-HTTLPR polymorphism with various behavioral phenotypes (see Dobson and Brent, 2013; Bevilacqua, 2018). Some authors have proposed that the observed effects of 5-HTTLPR may be influenced by interactions with environmental factors (Homberg and van den Hove, 2012), possibly through epigenetic modifications (Grünblatt et al., 2018).

As a general tendency, several candidate gene studies, as well as gene x environment studies, have failed to replicate the associations reported in previously published research. While significant results are more likely to be published than failed attempts, as discussed by Duncan and Keller (2011), despite the absence of a publication bias in present meta-analysis, the absence of association here documented is probably underestimated due to the existence of undisclosed observations.

In the context of AN, conducting additional studies, particularly focusing on Chinese and Japanese individuals, could help elucidate a role for this polymorphism in sufficiently representative cohorts and enlarge our understanding of potential geographic or population influences so far excluded by existing reports.

With reference to the apparently contradictory risk role attributed to the S allele we mentioned above, it is important to acknowledge that the transcriptional difference between the

S and L alleles, also defined as "low functioning" and "high functioning", respectively, has primarily been demonstrated in vitro using reporter constructs in non-neural cells (Iurescia et al., 2016). Therefore, further investigation is warranted to ascertain whether the 5-HTTLPR polymorphism exerts differential effects in vivo. This could be influenced by central nervous system-specific regulatory pathways, including epigenetic mechanisms (Grünblatt et al., 2018), interactions of the 5-HTT promoter with other regulatory regions, and potential feedback control loops. Post-mortem expression studies may provide valuable insights to reconcile this apparent paradox.

Conclusions

While the involvement of 5-HTT in the etiology on AN has long been hypothesized, our review and meta-analysis of fifteen candidate gene studies do not provide evidence supporting an association between the 5-HTTLPR polymorphism and AN.

At present, it seems improbable to attribute a role in the development of AN to a single serotonin gene polymorphism. To clearly exclude such a role, additional investigation and larger samples would be necessary. While the apparent trend in candidate gene studies suggests that this goal will not be easily pursued, the use of a next-generation sequencing approach may provide growing opportunities in this field (David, 2021) and should encourage additional research.

Further insights into this issue are expected from analysis of the interactions among specific genes involved in the regulation of serotonin/monoamine function, as previously reported for 5-HTT, the norepinephrine transporter (Urwin et al., 2003) and monoamine-oxidase (Urwin and Nunn, 2005).

Indeed, a deeper understanding of the polygenic/multifactorial nature suggested by present information on AN and EDs in general will likely require genome-wide association studies and epigenetic research, respectively. Epigenetic studies could shed light on gene x environment interactions that may contribute to the etiologic roles of psychological, environmental, and social factors in these disorders.

In sum, no definitive clinical implications for AN can be drawn from individual serotonin gene polymorphisms considered in the present and other meta-analyses (Solmi et al., 2016; Bevilacqua et al., 2024). In a bio-psycho-social framework, we would therefore underscore the relevance of integrating studies on genetic, psychological, and environmental factors, to gain a more comprehensive understanding of the complex nature and early risk detection of the disorder.

Study limitations

We acknowledge some limitations of present meta-analysis due to inherent aspects of published studies, especially small sample sizes, in some cases patient and control groups substantially different in size, overall limited numbers of cases (2,021) and controls (2,232), and other features that allowed subgroup analysis only on a geographic basis. These limitations are, however, reduced with respect to previous meta-analyses that comprised smaller numbers of studies.

As for our rigorous search and selection strategy, it minimizes the likelihood that our results are limited due to possibly missing peer-reviewed publications, but excludes any other report type as possible source of additional data.

Finally, it is important to note that our meta-analysis did not differentiate between the binge eating/purging subtype and the restricting subtype of AN. Since this differentiation is considered in some studies but not all, increasing the numbers of genetic studies focused on these phenotype variants would allow to acquire deeper information on the disorder.

Ethics approval and consent to participate
Not applicable.

Availability of data and materials
Data used for the present meta-analysis are available from the corresponding author upon reasonable request.

Competing interests
All authors declare that they have no competing interests.

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Authors' contributions
DLP and AB conceived the study. FS and DLP independently performed the database search for articles and data collection. All authors performed article selection, quality, risk of bias and data check independently, FS and AB performed statistical analyses. AB wrote the article. All authors have double checked all references, have reviewed and approved the article.

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List of abbreviations

5-HT, serotonin
5-HT2A receptor, serotonin 2A receptor
5-HTR2A, serotonin 2A receptor-encoding gene
5-HTT, presynaptic serotonin transporter
5-HTTLPR, serotonin transporter-linked promoter region
AN, anorexia nervosa
BED, binge eating disorder
BMI, body mass index
BN, bulimia nervosa
CI, confidence interval
CINAHL, Cumulated Index to Nursing and Allied Health Literature
DSM, Diagnostic and Statistical Manual of Mental Disorders
EBSCO, Elton B. Stephens Company
ED, eating disorder
FE, fixed effects model
GWAS, genome-wide association study
HPA axis, hypothalamic-pituitary-adrenal axis
HW, Hardy-Weinberg
ICD, International Classification of Diseases
L, long 5-HTTLPR allele
NOS, Newcastle–Ottawa quality rating scale
OR, odds ratio
PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PROSPERO, International Prospective Register of Systematic Reviews
PTSD, post traumatic mood disorder
rs4795541, polymorphic region in human SLC6A4 or 5-HTTLPR
rs6311, single nucleotide polymorphism in human 5-HTR2A
S, short 5-HTTLPR allele
SLC6A4, Solute Carrier Family 6 Member 4, 5-HTT-encoding gene
SNP, single nucleotide polymorphism
SSRI, selective serotonin reuptake inhibitor
TDT, transmission disequilibrium test