Association of trait-defined, eating-disorder sub-phenotypes with (biallelic and triallelic) 5HTTLPR variations

Howard Steiger *, Jodie Richardson, Norbert Schmitz, Ridha Joober, Mimi Israel, Kenneth R. Bruce, Lise Gauvin, Cathy Dandurand, Annelie Anestin

Eating Disorders Program, Douglas University Institute in Mental Health, 6875 LaSalle Blvd., Montreal (Verdun), Quebec, Canada H4H 1R3

ARTICLE INFO

Article history:
Received 7 January 2009
Received in revised form 12 March 2009
Accepted 17 March 2009

Keywords:
Eating disorders
Latent classes
Serotonin
Genetics
5HTTLPR

ABSTRACT

Context: Efforts to classify eating-disordered individuals based on concurrent personality traits have consistently converged on a typology encompassing “over-regulated”, “dysregulated”, and “low psychopathology” subgroups. In various populations, evidence has associated personality variations of an “over-regulated/dysregulated” type with differences on serotonin-system indices, and specifically, with different loadings of serotonin transporter promoter regulatory region polymorphism (5HTTLPR) genotypes and alleles. We explored the extent to which an empirical, trait-defined typology of eating-disordered individuals coincided systematically with variations in 5HTTLPR, assayed using biallelic and triallelic models.

Method: We tested 185 women with a DSM-IV eating disorder (108 with Bulimia Nervosa, 17 Anorexia Nervosa, and 60 an Eating Disorder Not Otherwise Specified) and 93 with no eating disorder on measures reflecting psychopathological traits and 5HTTLPR (biallelic and triallelic) genotypes and alleles.

Results: The highest-function, triallelic (LA/LA) genotype occurred significantly more frequently among eating-disordered individuals than among controls. However, a more fine-grained analysis suggested that this association was attributable to the fact that, among eating-disordered participants, those displaying an “Inhibited/Compulsive” profile (derived using latent class analysis) were more likely than those of a “Dissocial/Impulsive” or a “Low Psychopathology” group to carry the triallelic 5HTTLPR gain-of-function LA allele and to be LA/LA homozygotes.

Discussion: This study’s empirically derived classes coincide with interpretable differences on genetic indices—associating an “Inhibited/Compulsive” group with 5HTTLPR gain-of-function LA allele and to be LA/LA homozygotes. The findings, furthermore, suggest that 5HTTLPR, by influencing personality-trait manifestations may, in turn, influence eating-disorder risk and symptom expression.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Evidence implies that concurrent psychopathological traits demarcate clinically relevant sub-phenotypes within the eating-disordered population. For example, factor- or cluster-analytic studies in the area yield consistent support for the occurrence of compulsive (“over-regulated”), impulsive (“dysregulated”), and “psychologically intact” eating-disorder (ED) subgroups (e.g., Westen and Harnden-Fischer, 2001; Steiger and Bruce, 2007; Wonderlich et al., 2005). Anorexic ED variants (especially those of the “restrictor” variety) tend to occur preferentially within the over-regulated subgroup, whereas bulimic variants, although heterogeneously distributed, tend to occur preferentially in the dysregulated subgroup. In eating- and non-eating-disordered populations alike, traits of a “compulsive” or “impulsive” type have been associated with definable variations in serotonin (5-hydroxytryptamine: 5-HT) system function (Cloninger et al., 1993; Hollander, 1998; Steiger and Bruce, 2007; Steiger, 2004).

1.1. Psychopathological correlates of 5-HT

The 5-HT system regulates mood, social behavior, impulsivity and eating behavior (Steiger, 2004)—creating an obvious rationale for the hypothesis that 5-HT has a role in ED pathogenesis. In support, studies in eating-disordered individuals document disorder-relevant alterations in 5-HT metabolism, receptor sensitivity and transporter activity (Steiger, 2004; Frank and Kaye, 2005). Using single photon emission computer tomography, studies have shown reduced central 5-HT transporter availability in women with BN
(Tauscher et al., 2001). Likewise, using platelet measures, studies have suggested altered peripheral 5-HT reuptake in active anorexics and bulimics (Bruce et al., 2006; Steiger et al., 2005a), in binge-purge free former bulimics (Steiger et al., 2005) and even in ED patients’ unaffected relatives (Steiger et al., 2006). All of the preceding implicate altered 5-HT transporter kinetics in ED pathogenesis. Consistent with this notion, some candidate-gene studies associate low-function alleles of the 5-HT transporter promoter polymorphism (5HTTLPR)—called “low function” because they are associated with lower levels of transcription of the transporter protein—with the EDS (Di Bella et al., 2000; Matsushita et al., 2004). Association findings are, however, inconsistent (cf., Monteleone et al., 2006; Steiger et al., 2005b).

One basis for inconsistent association may be that 5HTTLPR, rather than conveying direct risk for ED development, influences the expression of behavioral traits that indirectly impact susceptibility to an ED. In non-eating disordered populations, data have supported the idea that traits of compulsivity and impulsivity may have different rates of coincidence with 5HTTLPR low- and high-function alleles and genotypes. The low-function alleles have been associated with impulsivity (Lesch et al., 1996), novelty seeking (Sander et al., 1998), affective instability and suicidality (Anguelova et al., 2003)—all arguably characterized by “dysregulation”—whereas the high-function alleles have been linked to obsessive-compulsive disorder (OCD; Hu et al., 2006; Baca-Garcia et al., 2005; Bengel et al., 1999) or “hyperfrontality” (Heinz et al., 2005)—both, arguably, associated with cortical “over-regulation”. Suggesting that the same tendency may exist in an eating-disordered population, recent studies have shown that bulimic individuals who carry low-function 5HTTLPR alleles are more likely to display traits of affective instability, impulsivity (Steiger et al., 2005b), sensation seeking (Steiger et al., 2007), or harm avoidance (Monteleone et al., 2006).

Despite the preceding, a recent study implicating 178 bulimic women found no association between 5HTTLPR variations and latent profile analysis-derived personality clusters characterized as “low psychopathology”, “affective perfectionistic” and “impulsive” (Wonderlich et al., 2005). We felt a replication to be warranted, partly in view of findings (noted earlier) showing positive association between 5HTTLPR variants and personality traits, partly because the study’s sample included a disproportionate number of low-function allele carriers, and partly because the study relied upon a potentially imprecise “biallelic” conceptualization of 5HTTLPR (explanation to follow).

1.2. 5HTTLPR: biallelic or triallelic?

The 5HTTLPR polymorphism has (for many years) been conceptualized as being biallelic, with long (L) and short (S) allele variants thought, respectively, to correspond to relatively high or low production of 5-HT transporter protein (Lesch et al., 1996). However, recent data suggest the existence of a low-frequency L-allele variant, L_c (an L allele with A → G SNP in its sequence) whose functioning seems to be akin to that of the S allele (Hu et al., 2006; Zalsman et al., 2006). Such data imply that 5HTTLPR may need to be conceptualized as being triallelic, with S and L_c alleles being comparable “low-function” variants, and an L_A allele conferring gain-of-function. Recent findings have, furthermore, associated the triallelic gain-of-function allele with obsessive-compulsive disorder (OCD), showing the L_A/L_A genotype and the overall frequency of the L_A allele to be substantially increased in individuals with OCD (Hu et al., 2006). Given an existent literature based on a biallelic 5HTTLPR assay, and new evidence that the polymorphism may be triallelic, we opted to examine effects of biallelic and triallelic 5HTTLPR formulations.

1.3. The present study

Our first goal was to develop an empirical classification of eating-disordered individuals based on assessment of variations along theoretically indicated, comorbid psychopathological traits. Following from previous studies (e.g., Westen and Harnden-Fischer, 2001; Wonderlich et al., 2005), we anticipated finding “over-regulated” (compulsive), “dysregulated” (impulsive) and “low psychopathology” sub-phenotypes. Subsequently, we planned to examine associations between empirically-derived sub-phenotypes and 5HTTLPR variants. The available literature led us to anticipate that the “compulsive” subgroup might show stronger loadings of “high-function” (L_c in the triallelic model, or L in the biallelic model) 5HTTLPR alleles than would other eating-disordered or normal-eater groups, whereas the “impulsive” subgroup might show stronger loadings of the “low-function” (L_c and/or S) alleles.

2. Methods

2.1. Participants

All participants in this institutional ethics-board approved study differed from those assessed by Wonderlich et al. (2005). All gave informed consent. Eating-disordered participants were recruited through a specialized Eating Disorders (ED) program in Montreal, Quebec, Canada. Given that diagnostic heterogeneity suited our interest in the spectrum of traits found in a broad, eating-disordered population, we included individuals with the DSM-IV ED diagnoses Anorexia Nervosa (AN), Bulimia Nervosa (BN) and Eating-Disorder Not Otherwise Specified (EDNOS). Our eating-disordered sample consisted of 185 women, 99 (53.5%) meeting DSM-IV criteria for BN-Purging (BN-P) subtype, 47 (25.4%) for BN-Nonpurging (BN-NP) subtype, 9 (4.9%) for AN Restricting (AN-R) subtype, 8 (4.3%) for AN binge-eating/purging (AN-BP) subtype, 47 (25.4%) for a bulimia-spectrum EDNOS (EDNOS-BN), and 13 (7.0%) for an anorexia-spectrum EDNOS (EDNOS-AN/R or EDNOS-AN/BP). EDNOS disorders were defined as follows: subjects (n = 47) with BMI of 18 or more who binged or purged, but at less than the requisite twice weekly (on average over the past 3 months), were regarded as having a BN-spectrum EDNOS (EDNOS-BN); individuals (n = 7) who had lower body weight, and engaged in regular binging and/or purging, but either failed to meet AN criteria due to weight above BMI of 17.5 or presence of menses were classified as having an AN Binge/Purge spectrum EDNOS (EDNOS-AN/BP); individuals (n = 6) who engaged in restriction and/or excessive exercise without binging or purging, but who failed to meet AN criteria due to weight above BMI of 17.5 or presence of menses were classified as having an AN Restricting spectrum EDNOS (EDNOS-AN/R). When interested in comparing anorexic versus bulimic disorders, we compared AN/R, AN/BP, EDNOS-AN/R and EDNOS-AN/BP groups to BN and EDNOS/BN groups. When interested in comparing “restrictors” to “bingers/purgers”, we compared AN/R and EDNOS-AN/R to BN, EDNOS/BN, AN/BP and EDNOS-AN/BP groups. A major part of the sampling for this study was conducted through a project concerned with bulimia-spectrum disorders (to which consecutive, consenting patients with such eating syndromes were recruited), with patients with Anorexia Nervosa added in an ad hoc way. Therefore, proportions of cases with different diagnoses achieved a desired degree of heterogeneity as to ED diagnoses, but do not reflect actual patterns of referral to our program.

We also recruited 93 normal-eater control women, drawn from an age group comparable to that of our ED sample, and with recruitment through public media and school-based announcements, so as to produce a group that included comparable propor-
tions of student and non-student participants to those found in our ED sample. To be eligible for the normal-eater group, participants had to be free of clinical ED symptoms according to the EDE, of a history of ED according to initial screening, and to have BMI between 18 and 34. So as not to skew the sample towards super-normalcy, we accepted 8 normal-eaters who emerged, on the structured interviews described below (see Section 2.2) as having had an Axis-I disorder within the past 12 months. Disorders detected included major depressive disorder ($n = 4$), social phobia ($n = 2$), post-traumatic-stress-disorder (PTSD; $n = 2$), and cannabis dependence ($n = 2$).

Subjects were between the ages of 17 and 50 (mean = 25.92 ± 7.05 for eating-disordered subjects and 24.43 ± 6.24 for controls). Body Mass Index (BMI: kg/m$^2)$ fell between 12.5 and 34 for ED subjects (mean = 21.39 ± 3.89) and 18 and 34 for control subjects (mean = 22.00 ± 2.55). Predictably, AN-spectrum (AN-R, AN-BP and EDNOS-AN) cases had a significantly lower mean BMI (mean = 16.93 ± 1.86) than did BN-spectrum (BN-P, BN-NP EDNOS-B) cases (mean = 22.26 ± 3.58) and normal-eater controls (mean = 22.00 ± 2.55) [$F = 37.87; df = 2, 275; p = .000]$. AN-spectrum, BN-spectrum and control cases did not, however, differ as to mean Age. Limiting recruitment to unmedicated individuals was impractical (and undesirable on grounds of representativeness), and we therefore included 77 women (41.6% of the eating-disordered sample) and 1 normal-eater control who were taking a psychoactive medication when screened for comorbid (past 12 months) DSM-IV Axis-I disorders in control subjects was accomplished using the Structured Clinical Interview for DSM-IV Axis-I disorders (SCID-I: First et al., 1996), a computer-guided, interview-based version of the Diagnostic Interview Schedule, Version IV (DIS4: Bucholz et al., 1991), and/or the Clinician-Administered Post-Traumatic Stress Disorder Scale (CAPS: Blake et al., 1995) – all “industry standard” measures, exhibiting excellent reliability, and convergent and discriminant validity. (Variations in interviews applied reflected shifts in study protocols occurring during the patient recruitment reported here). Elsewhere, we have evaluated agreement between DIS4 and SCID-I diagnoses, and obtained excellent Kappas (and percent agreements) for past 12-month presence of Axis-I disorders (Steiger et al., 2006).

2.3. Genotyping

DNA samples, obtained from whole blood, were amplified by polymerase chain reaction (PCR) in a total volume of 20 µl, which contained 100 ng of genomic DNA, 200 µM of dNTPs, 10 pmol each of the forward and reverse primer, 1 U of Taq DNA Polymerase (Qiagen, Alameda, CA), 1 × PCR buffer, and 1 × Q solution (Qiagen). The forward primer (5′-ATG CCA GCA CCT AAC CCC TAA TGT-3′) and reverse primer (5′-GG ACC GCA TGG CCG GGA-3′) were used to amplify a region encompassing 5HTTLPR; long and short alleles were then resolved on a 2% agarose gel. The PCR protocol involved preheating the samples at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C (30 s), annealing at 64°C (30 s), and extension at 72°C (45 s), as well as a final hold of 5 min at 72°C. The $L_C$ and $L_A$ alleles were subsequently studied by enzymatic digestion of 7 µl of the above mentioned PCR product using 5 U of MspI and incubating at 37°C for a minimum of 3 h. The $L_C$ and $L_A$ alleles were then resolved on a 2% agarose gel.

2.4. Statistical analysis

The latent structure of psychopathology in individuals with EDs was examined using latent class analysis (LCA) applied to scores for the four higher-order DAPP dimensions, the BIS and the CES-D. LCA models associations between observed variables and a non-observable (latent) variable, aiming to identify the smallest number of latent classes that adequately describes associations among dimensions entered. Models were fitted by means of an Expectation Maximization (EM) algorithm, with the program Latent Gold 3.0 (Vermunt and Magidson, 2000). To identify the best-fitting model, we compared successive models by the Bayesian information criterion (BIC), Akaike information criterion (AIC), and percentage classification error. BIC and AIC information into four higher-order personality dimensions, based upon previously established factor analyses conducted on data from large general, personality-disordered and twin samples (Livesley et al., 1992; Bagge and Trull, 2003). The studies in question support the validity of the higher-order dimensions Emotional Dysregulation (onto which load DAPP-BQ subscales measuring Anxiousness, Identity Problems, Social Avoidance, Affective Lability, Cognitive Distortion, Oppositionalism, Submissiveness, Insecure Attachment, Suspiciousness and Narcissism), Dissocial Behavior (encompassing Stimulus Seeking, Conduct Problems, Rejection, and Callousness), Inhibition (including Intimacy Problems and Restricted Expression) and Compulsivity (including the Compulsivity subscale alone). To complement our assessment, we added the Barrat Impulsivity Scale (BIS, version 11: Patton et al., 1995) and the Centre for Epidemiological Studies Depression (CES-D: Weissman et al., 1977), both widely-known and validated for the measurement of the intended constructs.

Using the Dimensional Assessment of Personality Pathology—Basic Questionnaire (DAPP-BQ: Livesley et al., 1992). The DAPP-BQ is an empirically derived, self-report measure that systematically describes personality traits using 290 items, organized into 18 “trait” subscales. In the present study, DAPP subscales were aggregated among dimensions entered.
The document discusses the application of latent class analysis (LCA) to identify subgroups within a population based on their behavioral characteristics. The analysis revealed three distinct classes, which were labeled based on their profiles: Dissocial/Impulsive, Low Psychopathology, and Inhibited/Compulsive. These classes were characterized by different levels of emotional reactivity, dysregulation, and depression. The document also mentions the use of Chi-Squared tests and ANOVAs to compare different groups and the significance of medication use in these subgroups.

### 3. Results

#### 3.1. Latent class analysis

Criteria indicated a 3 latent-class model to provide best fit to our data (BIC values: 1 class: 4406.33; 2 classes: 4320.04; 3 classes: 4308.96; 4 classes: 4328.17; 5 classes: 4352.35; 6 classes: 4391.41). Analysis of classification errors also supported a 3-class model. To rule out potentially confounding effects of ED diagnosis on model estimation, we re-ran the LCA twice, once with a covariate differentiating individuals with AN-spectrum disorders (AN-R, AN-BP, EDNOS-AN/R or EDNOS-AN/BP) from those with BN-spectrum disorders (BN-P, BN-NP, or EDNOS-B), and a second time with a covariate differentiating individuals who binged and/or purged (i.e., with AN-BP, EDNOS-AN/BP, BN-P, BN-NP, or EDNOS-B diagnoses) from those who did not (i.e., AN-R or EDNOS-AN/R diagnoses). Although the covariate always emerged as a significant predictor of classification, both analyses yielded best-fitting 3-class solutions that differed in no substantive way from the original (no-covariate) analysis. (For brevity’s sake, we report results from the original analysis here.)

Table 1 shows means on the six scale scores entered into the LCA. Three classes were indicated which, in decreasing order of probability was the highest). Once formed, LCA-based groups were compared for ED diagnoses and symptoms using either ANOVAs or Chi-Squared tests as appropriate. In addition, frequencies of genotypes and alleles were compared across eating-disordered groups (organized to reflect diagnostic and trait differences of interest) and normal-eater groups using Chi-Squared tests.

### 3.2. Clinical features

Table 2 shows results of Chi-Squared tests (and frequency values) comparing the 3 LCA-based classes with respect to ED diagnoses (AN-spectrum disorder, Restricter subtypes, past history of AN) and medication use. In addition, ANOVAs (and mean values) for Age, BMI, Binge Days per Month, and Monthly Binge, Vomit and Purge Frequencies (the latter including episodes of vomiting, or laxative or diuretic misuse) are provided. Chi-squared tests indicated a difference across classes with respect to AN-spectrum and Restricter diagnoses, and a trend with respect to history of AN spectrum disorders, with the Inhibited/Compulsive class always including largest proportions of AN-spectrum (AN-R, AN-BP and EDNOS-AN) diagnoses, Restricter (AN-R and EDNOS-AN/R) subtypes, or past AN disorders. A final chi-squared analysis indicated members of the Low Psychopathology class to receive significantly less psychoactive medication than did members of either the Dissocial/Impulsive or Inhibited/Compulsive classes. The preceding tendency helps confirm that lower psychopathological expression in the Low Psychopathology group was not due to confounding effects of medication (i.e., disproportionately higher medication use) that cancelled symptoms in this group.

One-way ANOVAs indicated no significant group differences as to Age. However, in line with the finding of an increased number of AN cases in the Inhibited/Compulsive class, there was a tendency for this class to have a lower mean BMI relative to that in other eating-disordered groups. Potential group differences (eating-disordered groups only) as to frequencies of binge and purge symptoms were examined using ANOVAs. Outliers on variables recording frequencies of binge, vomit and purge episodes were transformed to the mean plus two standard deviations and, due to deviations from normality, square-root transformations were performed. (Table 2 shows actual, rather than transformed, values since both analyses revealed similar results.) Again, in line with the finding of an increased number of AN cases in the Inhibited/Compulsive class, this class reported lowest monthly binge days and episodes. At a statistical trend level, the Inhibited/Compulsive group also reported fewer monthly purging episodes than did other groups.

### 3.3. Genetic variables

Treating 5HTTLPR in a conventional (biallelic) fashion, frequencies (and percentages) of S/S, S/L, and L/L genotypes, respectively occurring in 42 (22.7%), 84 (45.4%) and 59 (31.9%) of our eating-disorder participants and 17 (18.3%), 54 (58.1%) and 22 (23.7%) of our control participants, were in conformity with Hardy-Weinberg equilibrium [ED: $\chi^2(1) = 1.31$, n.s.; Control: $\chi^2(1) = 2.53$, n.s.]. With a triallelic model, frequencies (and percentages) of groups who were carriers of two, one or no “low-function” (i.e., S or L) alleles, 58 (31.4%), 80 (43.2%) and 47 (25.4%), respectively in ED participants, and 27 (29.3%), 53 (57.6%) and 12 (13.0%), respectively in controls, were also in conformity with Hardy-Weinberg equilibrium [ED: $\chi^2(1) = 3.23$, n.s.; Control: $\chi^2(1) = 3.09$, n.s.].

Our next data-analytic step aimed to detect any differences in genotype or allele frequencies that corresponded to the presence or absence of an eating disorder. To do so, we first compared genotype and allele frequencies occurring in the overall group of eating-disordered individuals to those obtained in normal-eaters (see Table 3). Analyses were set up to examine rates of biallelic genotypes and alleles (see Table 3a and b), triallelic genotypes and alleles (see Table 3c and d), and finally triallelic genotypes organized into low function homozygotes, heterozygotes, or high-function homozygotes, and triallelic allele frequencies when rates of low-function and high-function alleles were compared (see Table 3e and f). A significant difference emerged in the analysis contrasting carriers...
of the highest-function trilallelic genotype (i.e., LA/LA) to other genotypes, with eating-disordered participants displaying significantly higher rates of the LA/LA genotype.

To ensure that we had not obscured potential anorexic-bulimic differences, we repeated the set of analyses shown in Table 3, but with eating-disordered participants organized into those displaying anorexia- (i.e., AN/R, AN/BP, EDNOS-AN/R, and EDNOS-AN/BP) or bulimia- (BN and EDNOS/BN) spectrum disorders. These analyses yielded no significant effects for biallelic or triallelic 5HTTLPR models, and no within-ED subtype differences. Likewise, a parallel set of analyses that organized the eating-disordered participants into those displaying “restrictive” or “bulimic” ED variants yielded no significant effects on the set of genetic indicators.

Suspecting that the difference observed between eating- and non-eating-disordered groups may have reflected different loadings of psychopathological traits within these groups, our next data-analytic step explored the correspondence between psychological-trait variations, on the one hand, and genotype- or allele-frequency variations, on the other. Table 4 shows analyses designed to detect differences among frequencies (and percentages) of 5HTTLPR genotypes and alleles (biallelic and trilallelic models) for individuals in each of the LCA, psychopathological-trait defined groups, as well as for the overall Normal-Eater control group. The biallelic formulation yielded no significant results in either (a) a 3 × 4 chi-squared test of association between groups and genotypes, or (b) a 2 × 4 chi-squared test of associations between groups and allele frequencies (see Table 4a and b). With the trilallelic 5HTTLPR formulation, tests of association between discrete genotypes and groups (see Table 3c) and between allele (S, Lc, and La) frequencies and groups (see Table 4d) both yielded statistical trends \( \chi^2 = 21.95; df = 15; p = .109, \text{ and } \chi^2 = 12.25; df = 6; p = .057, \text{ respectively}. \) More importantly, tests of association between genotypes (organized to reflect homozygosity or heterozygosity for low- and high-function alleles) and groups (see Table 4e) or between low- and high-function allele frequencies and groups (see Table 4f) both yielded significant results \( \chi^2 = 15.45; df = 6; p = .017 \text{ and } \chi^2 = 9.67; df = 3; p = .022, \text{ respectively}. \) A main implication is that members of the Inhibited/Compulsive eating-disordered group tended to be more likely than were members of other groups to be carriers of one or two copies of the high-function allele (La).

More fine-grained analyses, conducted using individual Chi-squared tests to localize differences between groups indicated the following (always in relation to the trilallelic 5HTTLPR): there were significant pairwise group differences as to genotype frequencies obtained in the Inhibited/Compulsive group versus the Disso-

| Table 1 |
| Mean scores (and standard deviations) for each latent-class derived cluster of eating-disordered patients and for normal-eater controls on Emotional Dysregulation, Dissocial Behavior, Inhibition, Compulsivity, Impulsivity and Depression scales. Contribution of scale scores to cluster classifications is reflected by Wald test values (betas are equal among classes). One-way ANOVAs reflect differences between actual group means (including controls). Values with different letters in their superscripts differ at the p < .05 level or better on Tukey’s HSD tests. Variations in ns and dfs reflect isolated missing values. |

<table>
<thead>
<tr>
<th>Dissocial/Impulsive</th>
<th>Low psycho-pathology</th>
<th>Inhibited/Compulsive</th>
<th>Wald test p value</th>
<th>Normal-eater</th>
<th>F value, p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>M (SD)</td>
<td>M (SD)</td>
<td>M (SD)</td>
<td>M (SD)</td>
<td>df = 3, 274</td>
<td></td>
</tr>
<tr>
<td>Emotional Dysregulation</td>
<td>3.57* (0.39)</td>
<td>2.56* (0.44)</td>
<td>3.34* (0.34)</td>
<td>161.95 p &lt; .001</td>
<td>2.07* (0.48)</td>
</tr>
<tr>
<td>Dissocial Behavior</td>
<td>2.66* (0.52)</td>
<td>2.07* (0.38)</td>
<td>1.95* (0.42)</td>
<td>60.69 p &lt; .001</td>
<td>2.07* (0.36)</td>
</tr>
<tr>
<td>Inhibition</td>
<td>2.93* (0.71)</td>
<td>2.34* (0.57)</td>
<td>3.43* (0.59)</td>
<td>64.68 p &lt; .001</td>
<td>2.03* (0.58)</td>
</tr>
<tr>
<td>Compulsivity</td>
<td>3.41* (0.81)</td>
<td>3.18* (0.53)</td>
<td>4.23* (0.50)</td>
<td>66.34 p &lt; .001</td>
<td>2.97* (0.75)</td>
</tr>
<tr>
<td>CES-D depression</td>
<td>9.79* (7.63)</td>
<td>11.22* (9.55)</td>
<td>61.82* (9.55)</td>
<td>112.08 p &lt; .001</td>
<td>61.89* (9.55)</td>
</tr>
<tr>
<td>n = 32</td>
<td>n = 27</td>
<td>n = 23</td>
<td>n = 93</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Table 2 |
| Frequencies of diagnoses (AN-spectrum and Restricter), diagnostic history (history of AN) and medication use, as well as means for Age, BMI and Binge Days per Month, and for Monthly Binge, Vomit and Purge Frequencies (including episodes of vomiting, and laxative or diuretic misuse) by LCA-based groups. Chi-Square and F values reflecting inter-group difference are also reported. Values with different letters in their superscripts differ at the p < .05 level or better on Turkey’s HSD tests. |

<table>
<thead>
<tr>
<th>Dissocial/Impulsive (n = 80)</th>
<th>Low psycho-pathology (n = 73)</th>
<th>Inhibited/Compulsive (n = 32)</th>
<th>Statistical test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (%)</td>
<td>Frequency (%)</td>
<td>Frequency (%)</td>
<td>Chi-Squared df = 2</td>
</tr>
<tr>
<td>AN-spectrum</td>
<td>8 (10.0)</td>
<td>10 (13.7)</td>
<td>12 (17.5)</td>
</tr>
<tr>
<td>Restricter</td>
<td>2 (2.5)</td>
<td>6 (8.2)</td>
<td>7 (17.7)</td>
</tr>
<tr>
<td>History of AN</td>
<td>35 (44.3)</td>
<td>25 (35.7)</td>
<td>19 (59.4)</td>
</tr>
<tr>
<td>Psychiatric medication</td>
<td>39 (48.8)</td>
<td>22 (30.1)</td>
<td>16 (50.0)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>F value df = 2, 182</td>
</tr>
<tr>
<td>Age</td>
<td>24.70 (5.36)</td>
<td>26.77 (7.48)</td>
<td>27.06 (9.21)</td>
</tr>
<tr>
<td>BMI</td>
<td>21.30 (3.03)</td>
<td>22.06 (4.35)</td>
<td>20.11 (4.43)</td>
</tr>
<tr>
<td>Binge days/month</td>
<td>13.69* (9.74)</td>
<td>11.59* (9.40)</td>
<td>7.47* (9.57)</td>
</tr>
<tr>
<td>Binge episodes/month</td>
<td>23.52* (23.48)</td>
<td>18.82* (23.58)</td>
<td>11.31* (17.41)</td>
</tr>
<tr>
<td>Vomit episodes/month</td>
<td>34.21 (43.50)</td>
<td>27.51 (42.87)</td>
<td>18.72 (28.46)</td>
</tr>
<tr>
<td>Purge episodes/month</td>
<td>42.03 (46.38)</td>
<td>31.29 (43.09)</td>
<td>23.48 (28.42)</td>
</tr>
</tbody>
</table>

| Notes | Wald test values pertain only to eating-disordered participants, who were included in the latent class analysis. |

Of the highest-function trilallelic genotype (i.e., LA/LA) to other genotypes, with eating-disordered participants displaying significantly higher rates of the LA/LA genotype.
The main goal of the present study was to explore the association of 5HTTLPR variations with presence of an eating disorder, and with trait profiles that characterize eating-disorder sufferers. Although we found the highest-function 5HTTLPR genotype (LA/LA) to be more common in eating-disorder sufferers than in controls, finer-grained analyses examining associations of genetic variations with variations in psychopathological traits suggested that the association observed was not so much characteristic of our eating-disordered sample (in a wholesale fashion), or of particular diagnostic subgroups (e.g., those with Anorexia-spectrum syndromes), as it was of those members of the sample who were markedly Inhibited/Compulsive. In other words, our results associate genetic variations more specifically with trait variations (such as the Inhibited/Compulsive versus Dissocial/Impulsive distinction we derived) than with presence or absence of an ED syndrome per se. In this respect, our results corroborate other findings (obtained in eating- and non-eating-disordered populations) suggesting that heightened inhibition/compulsivity coincides with the gain-of-function (LA) allele of 5HTTLPR (Hu et al., 2006), and heightened impulsivity and affective instability with the low-function 5HTTLPR alleles (Anguelova et al., 2003; Lesch et al., 1996; Sander et al., 1998; Steiger et al., 2005b, 2007). Our study may, furthermore, take a step beyond the simple corroboration of the association noted, in suggesting a correspondence between the polymorphism of interest and phenotypic variations (or latent classes) that are validated empirically—our hope being that this effort may improve the stability (and hence replicability) of the findings. Members of an empirically derived “Inhibited/Compulsive” subgroup (when compared to those of Dissocial/Impulsive or Low Psychopathology ED subgroups, or members of a normal-eater control group) were significantly more likely to carry at least one copy of the triallelic 5HTTLPR gain-of-function LA allele, and to be high-function homozygotes (i.e., LA/LA genotype carriers) – which for 5HTTLPR represents the highest-expressing genotype.

In a similar vein, “Inhibited/Compulsive” group members were more likely than other LCA-based groups to exhibit Anorexia Nervosa (versus a bulimic ED variant). Important clues as to the interpretation of this finding would seem to lie in the observation that the LA allele is, elsewhere, associated with heightened risk of obsessive–compulsive disorder (Hu et al., 2006), and that obsessive–compulsive characteristics predominate in anorexic (and particularly restrictive-anorexic) ED variants (Westen and Harnden-Fischer, 2001; Steiger and Bruce, 2007). Together, these observations suggest that the 5HTTLPR LA allele, while not a specific factor in eating-disorder risk, may exercise a “pathoplastic” effect—heightening the likelihood of expression of obsessive–compulsive phenomena and pronounced dietary restraint in eating-disordered individuals. In other words, we may be observing a shaping impact of a genetic factor (in this case, of the 5HTTLPR high-function alleles) upon expression of traits (in the Inhibited/Compulsive versus Dissocial/Impulsive spectrum) which, in eating-disorder sufferers shapes eating-symptom expression (i.e., anorexic versus bulimic forms). In addition, our results support the notion that Anorexia Nervosa may not only resemble obsessive–compulsive disorder in a phenomenological sense (bodily obsessions motivating dieting compulsions), but that these disorders may also have at least one common, molecular-genetic determinant (i.e., the 5HTTLPR LA allele).

On the “opposite side of the same coin”, we note that members of our Dissocial/Impulsive subgroup were significantly more likely than members of the “Inhibited/Compulsive” group to carry low-function genotypes and alleles. The preceding corroborates parallel findings obtained in eating-disordered (Steiger et al., 2005b, 2007) and non-eating-disordered populations (Lesch et al., 1996;
How should such findings be interpreted? Evidence of lack of association of the triallelic 5HTTLPR with PET-determined transporter binding in normal humans has led to the proposal that associations of 5HTTLPR with clinical phenotypes may depend, not upon direct (momentary) effects on serotonin binding, but effects of 5HTTLPR upon brain development (Parsey et al., 2006). Follow-up direct (momentary) effects on serotonin binding, but effects of potential interest. Furthermore, in this study, we have not addressed gene–environment interaction effects that may be relevant to the understanding of associations between genetic factors, on the one hand, and psychopathology-defined classifications, on the other. Suggesting that such effects may be important, our group has recently documented several instances of gene-environment interaction effects linking 5HTTLPR and childhood abuse to other phenotypes in a putative obsessive–compulsive spectrum (e.g., Hollander, 2007). Awaiting this clarification, we note that OCD traits and syndromic OCD are both common in eating-disordered patients (Westen and Harnden-Fischer, 2001; Steiger and Bruce, 2007).

### 4.1. Limitations

Our sample is relatively small for a multivariate exploration, and this may limit stability of findings and power to detect certain effects of potential interest. Furthermore, in this study, we have not addressed gene–environment interaction effects that may be relevant to the understanding of associations between genetic factors, on the one hand, and psychopathology-defined classifications, on the other. Suggesting that such effects may be important, our group has recently documented several instances of gene-environment interaction effects linking 5HTTLPR and childhood abuse to other traits as novelty seeking (Steiger et al., 2007) or Dissocial Impulsive personality disorder (OCPD, and other phenotypes in a putative obsessive–compulsive spectrum (e.g., Hollander, 2007). Awaiting this clarification, we note that OCD traits and syndromic OCD are both common in eating-disordered patients (Westen and Harnden-Fischer, 2001; Steiger and Bruce, 2007).

#### 4.2. Conclusions

Our findings provide evidence of association amongst trait-based, eating-disorder sub-phenotypes (inhibited/compulsive, dissociative, or low psychopathology) and variations in 5HTTLPR genotypes- and less so, with categorical ED phenotypes (e.g., Anorexia- versus Bulimia-spectrum syndromes). We believe...
that this pattern of findings may guide the understanding of the relationships between genotypic variations and complex phenotypes, like eating disorders and their subtypes. A “lesson” to be derived from the present findings may be that the correct loci at which to seek correspondences between genotypes and phenotypes may be those linking genetic effects to higher-order, trait-based phenotypes (e.g., Inhibited/Compulsive) rather than to syndromic variations (e.g., Anorexia Nervosa). Findings such as the present ones suggest, for example, that 5HTTLPR (and presumably other polymorphisms in the 5-HT system) may influence psycho-pathological and behavioral traits, and in so doing, may indirectly channel vulnerable individuals’ thinness and body-image preoccupations into either restrictive or bulimic expressions. This interpretation is consistent with general findings on serotonin indices in eating-disordered individuals suggesting that serotonin-system variations may be more powerful correlates of generalized psycho-pathological-trait variations seen in ED sufferers than they are of eating-symptom variations and severities per se (see Steiger, 2004; Steiger and Bruce, 2007).

Contributors
Howard Steiger was Principal Investigator (PI) on the grants that supported this research and on the research itself, and oversaw all aspects of this work. Jodie Richardson assisted in the study’s conceptualization and design, and conducted main data analyses. With Steiger, she helped co-write the first draft of this manuscript. Norbert Schmitz contributed to statistical aspects of the report, carried out data analyses, and offered particular guidance on latent class analyses. Ridha Joober was responsible for genetic assays and contributed to the conceptualization and design of the study. Mimi Israel contributed to the conceptualization and design of the study, and supervised clinical assessments and medical acts. Kenneth Bruce contributed to conceptualization and design of the study, to data analyses, and to training and supervision of research assistants. Lise Gauvin contributed to the conceptualization and design of the study, and to the design and execution of data analyses. Cathy Dandurand and Annelie Anestin contributed to participant recruitment, testing, data entry and management, literature searches, and design and execution of certain data analyses. All authors have contributed to different sections of draft manuscripts, and all have approved the final manuscript.

Role of funding sources
Funding agencies had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report, or in the decision to submit the paper for publication. Preliminary results from this study were presented at the annual meeting of the Eating Disorders Research Society, Montreal, Quebec, Canada, Sept. 26, 2008. Authors are grateful to Patricia Groleau, Catherine Villenneuve-Tang and Catherine Senechal for their assistance in various aspects of this research.

References


