Gene 654 (2018) 43–48

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Effect of dopamine receptor D4 (*DRD4*) haplotypes on general psychopathology in patients with eating disorders

Guillermo Gervasini[a,*], Luz M. González[b], Carmen Gamero-Villarroel[c], Sonia Mota-Zamorano[a], Juan Antonio Carrillo[a], Isalud Flores[d], Angustias García-Herráiz[d]

[a] Department of Medical and Surgical Therapeutics, Medical School, University of Extremadura
[b] University General Hospital, Service of Clinical Analyses, Valencia, Spain
[c] Virgen del Puerto Hospital, Service of Clinical Analyses, Plasencia, Spain
[d] Eating Disorders Unit, Institute of Mental Disorders of Extremadura, Badajoz, Spain

ARTICLE INFO

Keywords:
DRD4
Dopamine
Polymorphism
Anorexia nervosa
Bulimia nervosa

ABSTRACT

Among the many candidate genes analyzed in eating disorder (ED) patients, those involved in dopaminergic functions may be of special relevance, as dopamine is known to play a significant role in feeding behavior, the distortion of body image, hyperactivity and reward and reinforcement processes. We aimed to determine the effect of functional polymorphisms and haplotypes in the Dopamine Receptor D4 (*DRD4*) gene on general psychopathological symptoms in ED patients. Two-hundred-and-seventy-three ED patients [199 with Anorexia Nervosa (AN) and 74 with Bulimia Nervosa (BN)] completed the SCL-90R inventory and were genotyped for four functional, clinically relevant *DRD4* polymorphisms: three variants in the promoter region [120-bp tandem repeat (TR, long vs. short allele), C-616G and C-521 T] and a variable number of tandem repeats (VNTR) in exon 3 (7R vs. non-7R allele). After correcting for multiple testing, none of the assayed polymorphisms were individually associated with SCL-90R results. Four *DRD4* haplotypes (**1–*4*) were detected in the patients with a frequency > 0.1. In the BN group, haplotype **2* (non7R-TR long-C-C) was associated with higher scores in the three global SCL-90R indices (GSI, PSDI and PST) after Bonferroni correction ($p \leq 0.01$ in all instances). Furthermore, carriers of this haplotype displayed higher scores (worst symptomatology) in *Somatization*, *Obsessive-Compulsive*, *Anxiety*, *Phobic anxiety*, *Paranoid ideation* and the test *additional items* (*p*-values for the differences between carriers vs. non-carriers ranging from 0.0001 to 0.0110). Certain combinations of *DRD4* variants may contribute to psychopathological features in BN patients.

1. Introduction

Anorexia nervosa (AN) and bulimia nervosa (BN) are severe eating disorders (ED) which very often present with general psychopathological symptoms that may be indicative of comorbidities. These comorbid disorders (depression, obsessive-compulsive disorder, etc.) are frequently as protracted and impairing as the ED itself (Miotto et al., 2010; Martinussen et al., 2017; Gazzillo et al., 2013). Whilst there is a certain overlap regarding the nature of these disorders between AN and BN patients (Martinussen et al., 2017), some studies report a higher proportion for cluster B disorders in BN and cluster C disorders in AN (Cassin and von Ranson, 2005; Bornstein, 2010).

Dopamine genetic pathways are progressively gaining interest in ED research, not only because they play a significant role in feeding behavior or in reward and reinforcement processes (Kontis and Theochari, 2012; Bello and Hajnal, 2010), but also because dopaminergic transmission is central in many of the associated comorbidities. In this regard, variability in one of the most studied dopaminergic polymorphic genes, Dopamine D4 Receptor (*DRD4*), is known to be related to general symptomatology shown by ED patients, such as novelty seeking, substance abuse, impulsivity, perfectionism, anxiety, depression or obsessive-compulsive disorder, among others (Gelernter et al., 1997; Munafo et al., 2008; Bachner-Melman et al., 2007; Perez-Edgar et al., 2014; Guo and Tillman, 2009; Taj et al., 2013; Garriock et al., 2006).

The most extensively investigated polymorphisms in the *DRD4* gene locus is a 48-bp variable number of tandem repeats (VNTR) located in exon 3. Most notably, the 7-repeat (7R) allele has been associated with

Abbreviations: AN, Anorexia Nervosa; BN, Buimia Nervosa; DRD4, Dopamine Receptor D4; ED, Eating Disorders; SCL-90R, Symptoms Checklist – 90R; TR, Tandem Repeat; VNTR, Variable Number of Tandem Repeats

* Corresponding author at: Department of Medical and Surgical Therapeutics, Medical School, University of Extremadura, Avda. de Elvas s/n, E-06005 Badajoz, Spain.
E-mail address: ggervasi@unex.es (G. Gervasini).

https://doi.org/10.1016/j.gene.2018.02.035
Received 25 January 2018; Accepted 12 February 2018
Available online 15 February 2018
0378-1119/

higher weight and energy intake (Fontana et al., 2015). The variant would lead to reduced binding affinity and receptor density for dopamine neurotransmission (Schoots and Van Tol, 2003) and hence the carrier would be more sensitive to reward reinforcement of food. Other studies, however, point to the opposite direction of the effect (Guo et al., 2006). In the *DRD4* promoter region, a 120-bp tandem repeat (TR) (rs4646984) generates either a short (S) or a long (L) allele, with the latter showing lower transcriptional activity (D'Souza et al., 2004; Kereszturi et al., 2007). The S-allele has been proposed as a risk factor for attention deficit (Kereszturi et al., 2007), whilst the L-allele has been related to paranoid symptoms (Lai et al., 2010). In addition, the CC genotype of the C-616G SNP (rs747302) has been associated with borderline personality traits in psychiatric patients (Nemoda et al., 2010). Finally, the C-allele of the C-521 T SNP has been linked to higher scores of novelty seeking and impulsivity (Munafo et al., 2008). The precise effect of these two last point mutations on dopaminergic signaling remains controversial (Okuyama et al., 1999; Kereszturi et al., 2006; Barr et al., 2001; Tei et al., 2016).

Several reasons have prompted us to carry out this study. First, the information on the role of *DRD4* variability in the ED setting is very scarce, covering mostly AN patients (Bachner-Melman et al., 2007; Gervasini et al., 2013). Second, psychopathological traits and comorbid personality disorders are often overlooked in genetic association studies on ED. We have previously shown in ED patients that these non-ED-specific traits can be influenced by variability in genes located in the brain (Gamero-Villarroel et al., 2015; Gamero-Villarroel et al., 2014). Our hypothesis is that variability in the *DRD4* gene could also have an impact on these symptoms and traits shown by ED patients. With these premises, the goal of the study was to identify associations between haplotypes in the *DRD4* gene, i.e. combinations of allelic variants in the four loci considered (48-bp VNTR, 120-bp TR, C-521T and C-616G), and general psychopathological symptoms present in ED patients. In addition, we aimed to investigate whether these associations were diagnosis-dependent, as our previous data indicate for other central genes (Gamero-Villarroel et al., 2015; Gamero-Villarroel et al., 2014).

2. Patients and methods

A total of 273 consecutive, unrelated ED patients (199 with AN and 74 with BN) were included in this study. Patients visited the Eating Disorder Unit of the Institute of Mental Disorders (Badajoz, Spain) and were interviewed and diagnosed by one psychiatrist and one psychologist using the ED section of the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, 4th ed. Diagnosis was later re-evaluated to comply with the new DSM-5 guidelines and was blind to genotype.

Patients were either self-referred to the Unit or were referred by their general practitioners because of indications of a possible ED (significant alterations in weight, presence of suggestive psychological features, etc.). Exclusion criteria for the study, determined upon screening, included dementia, mental retardation, schizophrenia, Turner's syndrome, other neurological disorders and underlying endocrine pathologies. All the participants were white Spanish female subjects living in the Health District of Badajoz (Southwest Spain). Mean age of the AN and BN patients at the time of the study was 18.9 ± 6.2 and 20.9 ± 8.1 years, respectively.

The study protocol was approved by the Bioethics and Biosafety Committee of the University of Extremadura and was conducted in accordance with the Declaration of Helsinki and its subsequent revisions.

2.1. Psychometric evaluation

The determination of general psychopathological features in ED patients was performed by the Symptom Checklist 90 Revised (SCL-90R). This is a widely used 90-item psychiatric self-reported inventory that evaluates in a 5-point rating scale a broad range of psychological problems and symptoms of psychopathology. The questionnaire consists of three Global Indices [Global Severity Index (GSI), designed to measure overall psychological distress; Positive Symptom Distress Index (PSDI), designed to measure the intensity of symptoms and Positive Symptom Total (PST), showing the number of self-reported symptoms]; nine primary symptom dimensions (*Somatization*, *Depression*, *Anxiety*, *Hostility*, *Phobic Anxiety*, *Paranoid Ideation* and *Psychoticism*) and a number of additional items (Derogaitis, 1977). This inventory, which has previously been validated in the Spanish population (Derogaitis, 2002), has showed sufficient item measurement invariance, being thus regarded as a useful tool for screening overall psychopathology in adolescent psychiatric patients (Rytila-Manninen et al., 2016).

2.2. Genotype analysis

A 5-ml blood sample was drawn from all participants and stored at −80 °C until genomic DNA purification, which was performed with a Qiagen blood midi kit (Qiagen Inc., Chatsworth, CA) according to the manufacturer's instructions. Previously described PCR-RFLP (restriction fragment length polymorphism) techniques were utilized for the identification of the four polymorphisms considered, namely *DRD4* 48bp-VNTR (Lichter et al., 1993), *DRD4* 120-bp TR (Seaman et al., 1999), *DRD4* C-521T (Mitsuyasu et al., 1999) and *DRD4* C-616G (Mitsuyasu et al., 1999). These four polymorphisms were selected on the basis of their reported impact on gene function/expression and/or their involvement in psychiatric conditions (Dsouza and Craig, 2008). In the case of the exon 3 VNTR polymorphism, which can produce several alleles, these were grouped according to the presence or not of the 7R-repeat allele (i.e. the resulting genotypes were non7R/non7R; non7R/7R and 7R/7R) as previously described (Fontana et al., 2015). Sequenced samples were used as negative and positive controls to rule out possible genotyping errors. In addition, samples with inconclusive results were confirmed by direct sequencing (ABI3700 DNA Analyzer; Perkin-Elmer/Applied Biosystems).

2.3. Statistical analyses

The comparison of the allele and genotype frequencies between different groups was performed with the Chi-square or Fisher's exact tests. Differences of quantitative variables between ED subgroups were assessed with Student's T or Mann-Whitney tests, as appropriate. The presence of individual SNPs was associated with the SCL-90R scores in each of the nine primary symptom dimensions, the additional items scale and the three global indices. Single-marker analyses were carried out by using logistic regression models adjusted for age using the *SNPassoc* R package (Gonzalez et al., 2007). This software is available at *https://cran.r-project.org/web/packages/SNPassoc/index.html* and can be added to the R environment to obtain descriptive statistics and exploratory analysis of missing values, calculation of Hardy-Weinberg equilibrium and analysis of associations based on generalized linear models (either for quantitative or binary traits). After correcting for multiple testing by the Bonferroni method (four haplotypes identified), differences were considered to be significant when p values were below 0.0125. We did not consider the different psychopathological scales to correct for multiple testing, as this procedure has been suggested to be too stringent to detect a moderate correlation with different endophenotypes in similar studies (Mercader et al., 2007).

We used the software *haplo.stats* (*http://mayoresearch.mayo.edu/mayo/research/biostat/*), also in the R environment, to conduct haplotype analyses. *Haplo.stats* is a score test from generalized linear models that searches for associations between haplotypes and disease parameters under the null hypothesis of no haplotype effect without any assumption about the mode of inheritance. Associations were carried out by logistic regression models adjusted by age. The haplotype lowest