Association study of variants in genes *FTO, SLC6A4, DRD2, BDNF* and *GHRL* with Binge Eating Disorder (BED) in Portuguese women

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ABSTRACT

A population based case-control study was conducted in Portuguese women with overweight/obesity to investigate the possible association of variants in genes *FTO*, *SLC6A4*, *DRD2*, *BDNF* and *GHRL* with binge eating disorder (BED). The distribution of seven polymorphisms was evaluated in 31 BED patients and 62 controls. No significant associations were found between polymorphisms and BED. Of interest, a markedly lower frequency of the *FTO* rs9939609 obesity risk A-allele was found in BED patients (0.290) in relation to the control group (0.402). Contrasting with anorexia nervosa and bulimia nervosa, our data suggest that rs9939609 A-allele has no potential role in BED.

Keywords: BED genetic susceptibility; *FTO* rs9939609 polymorphism; obesity

1. Introduction

Eating disorders (EDs), including anorexia nervosa (AN), bulimia nervosa (BN) and binge eating disorder (BED), resulting from the interaction between psychological risk factors, sociocultural influences and genetic predisposition, affect up to 5% of the population in Western countries (Mayhew et al., 2018).

BED is the most common of EDs (prevalence of 2%–4%), marked by regular overly excessive eating episodes not associated with regular inappropriate compensatory behaviours (Yilmaz et al., 2015). Patients are at high risk of being either overweight or obese. Although BED heritability estimates range from 41% to 57% (Mayhew et al., 2018), no robust genetic associations have been conclusively identified. Main association studies in BED involved candidate genes of the leptin-melanocortin pathway including the melanocortin-4 receptor (*MC4R*) (Valette et al., 2013), brain-derived neurotrophic factor (*BDNF*) (Monteleone et al., 2006a) and ghrelin (*GHRL*) (Monteleone et al., 2007); or genes involved in the brain reward-related systems including the serotonin transporter gene (*SLC6A4/5-HTT*) (Monteleone et al., 2006b) and dopamine D2 receptor (*DRD2*) (Davis et al., 2012). The fat mass and obesity-associated gene (*FTO*), which has been identified as an obesity locus in multiple genome-wide association studies (GWAS) (Loos and Yeo, 2014), yielded mixed findings regarding a possible association with AN or BN (Jonassaint et al., 2011; Muller et al., 2012; Castellini et al., 2017). To the best of our knowledge, studies addressing the possible association between *FTO* and BED are totally lacking.

We conducted a population based case-control study to investigate in a series of Portuguese women the possible association with BED of variants in genes *FTO*, *SLC6A4*, *DRD2*, *BDNF* and *GHRL*, previously studied in the context of EDs (Yilmaz et al., 2015; Mayhew et al., 2018).

2. Methods

2.1. Study population and measurements

A total of 93 unrelated women of Portuguese origin, aged between 20 and 58 years old (mean 41.52 years) were recruited for this study. Twenty four subjects were defined as being overweight (25 kg/m^2≤ BMI <30 kg/m^2; mean 27.94) and 69 had obesity (BMI ≥30 kg/m^2; mean 36.08). In particular, 31 women diagnosed with BED (20–57 years old; mean 39.10 years) were compared with a sex matched control group of 62 women (22–58 years old; mean 42.73 years) without signs of BED psychopathology.

Clinical data to define diagnosis of BED were collected through a face-to-face structured clinical interview (lead by five experienced clinical psychologists) and self-reported questionnaires assessing eating psychopathological symptoms. Out of the 93 females, 31 were diagnosed with BED using the DSM-5 criteria (American Psychiatric Association, 2013). The existence of BED was carried out using the Eating Disorders Examination (EDE) interview, the international well established interview for the assessment of the cognitive and behavioural symptoms of EDs (Fairbun et al., 2008), and the Binge Eating Scale (BES) total score as a complimentary criterion (assuming BES > 17 as the threshold for BED) (Duarte et al., 2015).

Written informed consent was obtained from all individuals prior to enrolment. The study was conducted in accordance with Declaration of Helsinki and was approved by the Ethics Committee of *Centro Hospitalar e Universitário de Coimbra* (CHUC).

2.2. Genotyping

Genomic DNA was extracted from peripheral blood samples with the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The *FTO* single-nucleotide polymorphism

(SNP) rs9939609 was genotyped by TaqMan allelic discrimination assay (C_30090620_10) (Applied Biosystems, Foster City, CA). Genotyping for the 43-basepair insertion/deletion serotonin transporter–linked polymorphic region (5-HTTLPR) was performed by Polymerase Chain Reaction (PCR), as previously described (Dias et al., 2015). The remaining polymorphisms were genotyped by PCR-restriction fragment length polymorphism (PCR-RFLP). The following primers and restriction enzymes (available on New England BioLabs) were used: *DRD2* rs1800497, 5'-GAACATCACGCAAATGTCCA-3' (forward), 5'-CCTTGCCCTCTAGGAAGGAC-3' (reverse), *Taq$^{\alpha}$1*; *BDNF* rs6265, 5'-AGAAGAGGAGGCTCCAAAGG-3' (forward), 5'-AAACATCCGAGGACAAGGTG-3', *NlaIII*; *BDNF* rs16917237, 5'-GCAAACTTAGCTTCTCTGGGATA-3' (forward), 5'-CTGTGAACCAAAGCAATGGA-3' (reverse), *MseI*. *GHRL* rs696217 was genotyped using *BsrI* as reported in Monteleone et al. (2007) and rs4684677 using *ScaI*, according Huang et al. (2017). Digested products were separated in 2.5% agarose gels.

2.3. Statistical analysis

Genotype and allele frequencies were estimated by gene counting and the Hardy-Weinberg equilibrium were achieved through an exact test. The overall association between genotypes and BED was tested by logistic regression, in the additive model, estimating *p*-values, odds ratio (OR) and 95% confidence intervals (CI). These statistical analyses were performed using the PLINK software (Purcell et al., 2007).

Distribution of quantitative variables was analysed using the Kolmogorov-Smirnov test. Anthropometric characteristics, biochemical data and neuropsychological test scores between BED patients and control subjects were compared using Student's *t* test for normally distributed variables and the nonparametric Mann-Whitney *U* test for

asymmetrically distributed variables. These statistical analyses were conducted using SPSS version 24.0 (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

Mean Binge Eating Scale scores were significantly higher for the BED group than for the controls (27.3 +/- 7.1 vs. 15.3 +/- 7.0; $p < 0.001$). There were no statistically significant differences between the two groups of subjects in age, weight, height, BMI, degree of obesity, or blood chemistries (glucose, cholesterol, high- and low-density lipoproteins, and triglycerides).

Allele frequencies for the analysed polymorphisms are detailed in Table 1. Genotyping success rate was 97.6% considering all polymorphisms and genotype distributions were in Hardy-Weinberg equilibrium. All genetic variations revealed similar frequencies to those found in 1000G for Europeans (Ensembl EUR), except for *DRD2* rs1800497 that showed a marked higher frequency in controls for the minor A-allele (0.308 vs. 0.188) suggesting that obesity could be associated with this polymorphism in the Portuguese population.

The logistic regression revealed no statistically significant associations with BED for any of the analysed polymorphisms ($p > 0.05$) (Table 1). As studies addressing the possible association between *FTO* and BED are totally lacking, we tested the possible association of rs9939609 with BED. Of interest, a markedly lower frequency of the *FTO* rs9939609 obesity risk A-allele was found in BED patients (0.290) in relation to the control group (0.402). The interest in the relationship between the *FTO* locus and pathogenesis of EDs is because of its high expression in the hypothalamus, the region of the brain that controls appetite and energy balance (Gerken et al., 2007). Previous studies reported that A-allele was associated with an increased vulnerability to both BN and AN

(Castellini et al., 2017; Muller et al., 2012). A study by Jonassaint et al. (2011) did not find any significant association of seven *FTO* SNPs with AN, however rs9939609 was not evaluated. Contrasting with previous data for AN and BN, our study suggest that rs9939609 A-allele is not associated with BED.

The involvement of serotonergic genes in EDs have been studied extensively because of the important role of serotonin in eating behaviour. Conflicting findings on the association of polymorphism 5-HTTLPR in the promoter region of serotonin transporter gene (*SLC6A4*) have been found for AN and BN (Yilmaz et al., 2015). Although preliminary results by Monteleone et al. (2006b) suggested that polymorphism 5-HTTLPR may contribute to BED susceptibility, our study revealed no such association ($p = 0.689$).

Dopamine is an important monoamine neurotransmitter involved in feeding behaviour; consequently dopaminergic genes have been broadly studied in the context of EDs (Yilmaz et al., 2015). Davis et al. (2012) found a possible role of *DRD2* Taq1A polymorphism in BED, that was not replicated in our study ($p = 0.278$).

The brain derived neurotrophic factor (BDNF) is a protein involved in appetite suppression and both *BDNF* rs16917237 and rs6265 polymorphisms were extensively analysed in context of EDs (Gamero-Villarroel et al., 2014; Yilmaz et al., 2015). Monteleone et al. (2006a) explored the rs6265 genotype frequencies in women with BED and results suggested that, although predisposing to a more severe binge eating behaviour, this polymorphism does not contribute to BED susceptibility. Our data is in concordance with this study showing no association between rs6265 and BED ($p = 0.711$). Although non-significant ($p = 0.138$), our study revealed the *BDNF* rs16917237 minor T-allele with a marked higher frequency in controls (0.217) in comparison with BED patients (0.129).

The haplotype analysis combining the two *BDNF* polymorphisms also revealed no association with BED.

GHRL polymorphisms have been studied in the context of EDs and conflicting findings have been reported for AN and BN (Yilmaz et al., 2015). Although the *GHRL* Leu72Met polymorphism yielded significant association with BED in a preliminary pilot study (Monteleone et al., 2007), no such association were observed in our study for both individual polymorphisms rs696217 (p = 0.644) and rs4684677 (p = 0.505), or in haplotype combination.

In conclusion, similar to a number of candidate gene studies that have been inconsistent, our study have not clearly confirmed for patients of Portuguese origin the involvement in BED of any of the analysed SNPs in genes *FTO*, *SLC6A4*, *DRD2*, *BDNF* and *GHRL*. This can be due to the small sample size, which has been stated as a common limitation to identification of genetic variants for EDs across most all studies. Moreover, positive associations reported in the literature have been interpreted with extreme caution. Contrasting with AN and BN, our findings suggest for the first time that the *FTO* rs9939609 obesity risk A-allele has no potential role in BED.

Conflicts of interest

There are no conflicts of interest.

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Table 1. Allele frequencies and association of the study polymorphisms in genes *FTO*, *SLC6A4*, *DRD2*, *BDNF* and *GHRL* with risk of BED psychopathology.

Gene	Variant	Alleles (1:2)	Common name	MAF (EUR)	*n* (total)	MAF (total)	HWE *p*-value	*n*	MAF (no BED)	*n*	MAF (BED)	OR (95% CI)	*p*
FTO	rs9939609	A:T	-	0.414	92	0.364	0.498	61	0.402	31	0.290	0.630 (0.332-1.197)	0.158
SLC6A4	-	S:L	5-HTTLPR	0.444*	89	0.421	0.664	59	0.432	30	0.400	0.882 (0.476-1.633)	0.689
DRD2	rs1800497	A:G	Taq1A	0.188	91	0.280	0.190	60	0.308	31	0.226	0.690 (0.354-1.347)	0.278
BDNF	rs16917237	T:G	-	0.221	91	0.187	0.728	60	0.217	31	0.129	0.505 (0.205-1.245)	0.138
BDNF	rs6265	T:C	Val66Met	0.197	91	0.192	1.000	60	0.200	31	0.177	0.859 (0.386-1.915)	0.711
GHRL	rs696217	T:G	Leu72Met	0.087	72	0.097	0.503	42	0.107	30	0.083	0.767 (0.249-2.360)	0.644
GHRL	rs4684677	A:T	Gln90Leu	0.075	75	0.027	1	44	0.034	31	0.016	0.456 (0.045-4.597)	0.505

Abbreviations: Alleles 1:2, Minor:Major; A, Adenine; T, Thymine; C, Cytosine; G, Guanine; S, short; L, long; MAF, Minor Allele Frequency; EUR, European; *n*, sample size; HWE, Hardy-Weinberg Equilibrium; BED, Binge Eating Disorder; 95% CI, 95% confidence interval; OR, odds ratio.

Logistic regression was used to compare genotype distribution between the BED group and controls without BED symptoms. The OR and *p*-values (asymptotic) were obtained for the minor allele under an additive genetic model.

* Frequencies according Dias et al. (2015).