Title: **Evidence of complex involvement of Serotonergic genes with restrictive and binge purge subtypes of Anorexia Nervosa**

Short Title – Anorexia and the Serotonergic system

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Abstract:

Objectives: There is mixed evidence of association of serotoninergic genes with anorexia nervosa (AN), but substantial evidence for the involvement of serotonergic mechanisms in appetite control. This study was designed to investigate possible associations between the two subtypes of AN (Restricting-RAN, and Binge-purging–BPAN) and polymorphisms within 5 genes encoding for proteins involved in the serotoninergic system.

Methods: In order to carry out this investigation we have conducted a case-control association study on 226 females meeting the criteria for AN, and 678 matched healthy females.

Results: Our data show a significant association between polymorphisms with the gene encoding HTR2A with both AN subtypes, an association between polymorphisms within the genes encoding HTR1D and HTR1B with RAN, and an association between polymorphisms within the gene encoding HTR2C with BPAN. No associations were found for any polymorphisms of the serotonin transporter gene. This outcome indicates a substantial and complex inter-relationship between serotoninergic genes and AN.

Conclusions: Given these data we hypothesis that the expression or control of expression of several genes of the serotoninergic system, and interactions between these genes, could exert considerable influence over the specific symptomatology of the subtypes of AN.

KEY WORDS: Eating Disorder; Anorexia nervosa; genetics; serotonin; serotonin recpetor

Introduction:

Anorexia nervosa (AN) is a severely debilitating disorder that affects primarily women (Hebebrand et al. 1996,Elfhag and Linne 2005) and has the highest mortality rate of any of the psychiatric disorders (Sullivan 1995,Steinhausen 2002). Occurring predominantly during adolescence (Halmi et al. 1979) the disease is characterised by a pathological obsession for thinness through the control of eating behaviour. Evidence from family and twin studies have suggested that both genetic and environmental components contribute to the development of AN (Sokol et al. 2009,Fairburn et al. 1999,Lilenfeld et al. 1998,Vandereycken and Pierloot 1981,Garfinkel and Garner 1982). The genetic component has been estimated through meta analysis of twin studies as contributing up to 76% of the susceptibility to AN (Treasure and Holland 1990,Klump and Culbert 2007). Although no large pedigree studies showing Mendelian inheritance have been reported to date, and despite the existence of a large number of published genetic studies, the genetic component of this complex disorder is still far from being understood. This is likely to be due in part to the relatively low prevalence rate of this disorder. A further difficulty could be the existence of separate sub-types of this disorder (American Psychiatric Association 1994), which are likely to have somewhat different genetic profiles.

The serotonin theory of satiety was proposed more than 30 years ago (Blundell 1977), and subsequent research by many authors has confirmed the main tenets of this approach (Blundell and Halford 1998,Halford and Blundell 2000,Halford et al. 2004). In general, the release or blockade of re-uptake of serotonin causes an inhibition of eating, and drugs such as fenfluramine, fluoxetine and sibutramine have all been shown to suppress food intake and cause weight loss in obese people (Halford

2006,Van der Ploeg 2000). Fluoxetine has further more been shown to result in reduce rates of relapse after inpatient weight gain in Anorexia nervosa (Kaye et al. 2001). Considerable research on the serotonin receptor subtypes has implicated the HTR1B and HTR2C subtypes in the suppression of appetite (Park et al. 1999). Animal studies have shown that the hypophagia induced by the serotonergic releasing drug fenfluramine can be antagonised by a highly selective HTR1B antagonist (Simansky and Nicklous 2002) injected directly into the parabrachial nucleus, and by pre-treatment with the selective HTR2C antagonist. The hypophagia induced by the sertraline can also be blocked by serotonin antagonists acting on HTR1B and HTR2C receptor (Lucki et al. 1988). In addition the HTR1B receptor agonist significantly suppressed food intake and modified satiety (Halford and Blundell 1996), and a similar effect was shown by the selective HTR2C receptor agonist (Smith et al. 2006). These animal studies were complemented by studies in humans indicating that the HTR1B/1D receptor agonist (Sumatriptan) decreases food intake in healthy women (Boules et al. 2000). The preferential HTR2C receptor agonist mCPP induces weight loss over a 2 week period in obese subjects (Sargent et al. 1997). Taken together, these studies strongly suggest the involvement of HTR1B and HTR2C receptors in the inhibition of food intake. Transgenic animal studies have shown that the HTR1B and HTR2C knock-outs cause over-consumption and weight gain (Clifton et al. 2003). In keeping with this body of evidence are specific findings indicating a disturbance of serotonin metabolism in eating disorders (Kaye et al. 2005,Kaye 1997).

More broadly, in the eating disorders field the evidence for serotonin dysregulation has been intensively reviewed (Brewerton 1995) and several studies have shown an alteration of serotonin neurotransmission in AN and Bulimia nervosa even after the

restoration of body weight and or recovery (Kaye et al. 1998,Frank et al. 2002). Furthermore it has been reported that elevated cerebrospinal fluid concentrations of 5-hydroxyindoleactic acid (5-HIAA) occur both during and after recovery from AN and BN (Kaye et al. 1991). Studies have gone on to show that in individuals recovered from AN and BN exposed to serotonin challenges show altered behavioural responses (Frank et al. 2001,Kaye et al. 2003,Smith et al. 1999). The serotonergic pathway has been shown to be involved in feeding, satiety, fasting, mood, anxiety, impulsivity, addiction, body image, perception and gender (Steiner et al. 1997,Brewerton 1995). Given the relationship of these behaviours to phenotypic expression it has been argued that there is clear evidence for dysregulation of serotonin systems in anorexia nervosa (Jimerson et al. 1990). The reduced dietary intake of tryptophan is unlikely to play a major role in altered serotonin metabolism as tryptophan can be provided through tissue catabolism (Favaro et al. 2000). Over 20 years ago dysregulation implied an inappropriate release or reuptake of serotonin around the synapse, or aberrant 5HIAA levels in the CSF. However with the discovery of multiple subtypes (between 15 and 19 depending on the criteria adopted) of the serotonin receptor clustering within 7 separate families, the notion of serotonin dysregulation has become more complex and can take many forms. The term dysregulation can now be applied to unusual combinations of different subtypes of serotonin receptors with serotonin playing a role in complex behaviours through an interaction with multiple receptors. Our approach to the understanding of the subtypes of anorexia nervosa is based on this receptor model of dysregulation.

Several studies have investigated possible associations between AN and polymorphisms within serotoninergic genes. There are multiple reports on the

serotonin transporter (SLC6A4) gene and AN (Gorwood et al. 2003). Specifically research has focused on a functional polymorphism in the 5' regulatory promoter region which consists of two common alleles which vary due to a 44 base pair insertion (Long L-allele) or deletion (Short S-allele). In the S-allele form functional studies have revealed a reduction in serotonin transporter gene expression and uptake. There has been mixed evidence for the association of this polymorphism and anorexia nervosa, an initial meta-analysis of 4 studies revealed that there is a moderated yet significant association with the S-allele and anorexia nervosa (Gorwood 2004). A more recent meta-analysis of 8 studies further supported the Gorwood study and showed a strong association with the S form of SLC6A4 and AN (Lee and Lin 2009). A number of studies have examined the role of polymorphisms within the gene encoding HTR2A receptors and genetic susceptibility to AN. The HTR2A promoter polymorphism -14385G/A has been shown to be associated with susceptibility to AN, although this finding is not consistent across all studies. A meta-analysis of 14 studies suggests that when all data are combined there is statistically significant evidence of an association (Gorwood et al. 2003). One of the possible explanations for the diversity of these findings is that many studies have classified patients with the generic diagnosis of AN whereas others have investigated the specific sub-types. Since it is likely that different allelic profiles and physiological processes underlie each specific sub-type, the use of phenotypic sub-types is likely to be more robust.

A whole genome linkage study has produced the first report of an association of the HTR1D receptor locus with RAN (Bergen et al. 2003). We have confirmed this association (Brown et al. 2007). Of the 4 studies that have investigated the gene encoding the HTR2C receptor, 2 have reported positive associations (Hinney et al. 1997, Nacmias et al. 1999, Karwautz et al. 2001, Hu et al. 2003). Despite the strong

evidence for the involvement of HTR1B receptors in the inhibition of appetite, there appear to be no studies on the link between AN and the gene encoding the HTR1B receptor (Although it may be associated with low weight in bulimia nervosa (Levitan et al. 2001).

Given these data we have hypothesed that polymorphisms within genes encoding major components of the serotonergic system play an important role in defining genetic susceptibility to AN. Moreover, it is possible that different patterns of serotonin receptor genes could distinguish the restricting from the binge-purging subtypes of AN. In the current study we have performed a systematic analysis of polymorphisms within the genes encoding for SLC6A4, HTR1B, HTR1D, HTR2A and HTR2C in a single cohort of well characterised individuals with DSM-IV sub-typed AN.

Methods and Materials:

Subjects

226 female Caucasian patients, registered at Yorkshire Centre for Eating Disorders (Leeds, UK) between 1998 and 2002. All patients recruited to this study had at some point been admitted to the centre for inpatient treatment however at the date of completing the study the majority were outpatients (n=176). For each patient recruited, 3 female British Caucasian individuals were used as controls (total control population n = 678). The control group was selected from a large data base of healthy individuals in the general population collected for comparison purposes in genetic studies. From this healthy population we selected women who were matched with the cases for month and year of birth; therefore there was a good match for age and

gender between the cases and controls. In addition the controls did not contain any individual with any clinical condition. Patients were diagnosed according to DSM-IV criteria for eating disorders by a consultant psychiatrist during clinical interview before being approached to take part in this study. Diagnosis was confirmed through the administration of the structured interview for Anorexia and Bulimia (Fichter et al. 1991, Fichter and Quadflieg 2001). Patients were classified into two groups identified as restricting AN (RAN) or binge-purge AN (BPAN). For this study patients had to meet the diagnostic criteria for current diagnosis (i.e. previous 12 months). We further extended this to ensure that no diagnostic crossover had occurred within the last 36 months, but it should however be noted that in those cases where the patient had less than 3 years diagnosis of AN this would not be possible and as such it is likely that they may undergo diagnostic crossover within the next few years. Based on this analysis of the 226 anorexia nervosa patients 122 were characterised as RAN and 104 were characterised as BAN. After complete description of the study to each subject, written informed consent was obtained. The study was approved by the Leeds United Hospital Trust Ethical Review Committee (submission number 01/085).

Group Definitions

Participants were dichotomised according to DSM-IV subtype classification. Reports of current age, current weight, lowest and highest weight and age of onset were also recorded. From these data BMIs were calculated (BMI = weight (kg) / height $(m)^2$) see table 1.

Table 1: Mean scores and standard deviation of group descriptive

	Current BMI (kg/m^2)	Lowest BMI (kg/m^2)	Highest BMI (kg/m^2)	Length of ED (years)	Current Age (years)
Restrict (RAN) (n = 122)	16.45 (±2.55)	13.53 (±2.42)	22.38 (±2.88)	10.9 (±8.06)	28.6 (±7.32)
Binge/Purge (BPAN) (n = 104)	18.1 (±3.49)	14.5 (±2.63)	24.11 (±4.11)	8.00 (±6.74)	27.5 (±6.40)

Table of mean BMI for participants divided by AN subtype along with age on completion of study and length of time since diagnosis with ED

Statistics

Departure from Hardy-Weinberg Equilibrium (HWE) in the control population was assessed for each polymorphism using a chi-square test. An exact HWE permutation test was performed if the HWE chi-square p-value was <0.05 and if at least one genotype cell had an expected count <5 (Zaykin et al. 1995). Polymorphisms with a HWE p-value (chi-square or exact) <0.005 in control subjects were excluded from analysis.

For each SNP, testing for association between alleles/genotypes and disease status was carried out using the fast Fisher's exact test (FET) procedure. The fast FET computes exact p-values for contingency tables using the network algorithm developed by Mehta and Patel (1983).

For each SNP two parameters are calculated: (1) an odds ratio (95% CI) for the "at risk allele" (the allele that appears more frequently in cases than controls); (2) an odds ratio for the "genotype" (determined by identifying the genotype that has the largest chi-square value when compared against the other 2 genotypes. For example, if a SNP has genotypes AA, Aa and aa, 3 chi-square association tests are performed: (a) AA vs Aa+aa, (b) Aa vs AA+aa and (c) aa vs AA+Aa. If test (a) yields the highest chi-square value, then an odds ratio is calculated for the AA genotype vs the Aa+aa genotypes combined). In some cases the genotype may result in an increased risk and in some cases a decreased risk. Where the odds ratio is below 1 this represents a decreased risk and when the odds ratio is above 1 this represents an increased risk. The allele data are always presented as the allele which increases the risk.

Odds ratios (OR) were constructed for the "at risk allele" and "genotype" according to the formula OR = $(n_{11} * n_{22}) / (n_{12} * n_{21})$, where n_{11} = cases with "at risk allele"/"genotype", n_{21} = cases without "at risk allele"/"genotype", n_{12} = controls with "at risk allele"/"genotype", n_{22} = controls without "at risk allele"/"genotype". In order to avoid division or multiplication by zero, 0.5 was added to each cell in the contingency table. 95% confidence intervals for the ORs were calculated as follows: lower limit = $OR*\exp(-z\sqrt{v})$, upper limit= $OR*\exp(z\sqrt{v})$, where z = 97.5th percentile of the standard normal distribution and v= s-allele$[1/(n_{11})] + [1/(n_{12})] + [1/(n_{21})] + [1/(n_{22})]$.

Power of the sample

The current study examines a population of 226 AN patients and 678 healthy control individuals. This single cohort represents one of the largest AN case-control populations studied to date and given a minor allele frequency of 0.1 and an expected effect size of between 1.6 and 2.6 this study has power of between 77% and 99% at the p=0.05 level.

Association analysis was performed for each of the described SNPs comparing cases versus controls; RAN versus controls; BPAN versus controls; and, RAN versus BPAN.

Multiple testing issues

The current analysis forms part of a much larger study of 42 candidate genes for anorexia nervosa (data to be published elsewhere). In total we tested 176 SNPs for association with anorexia nervosa and its subtypes. Due to the large size of the overall study and the number of statistical tests performed, we would expect to see a number of associations, with $p<0.05$, simply by chance. However, given the complex

nature of the disorder, coupled with the nature of a "candidate" gene study, it has been impossible to put an exact figure on how many false positives may have been generated. In order to reduce the number of false positives, a semi-conservative Bonferroni-type correction has been applied to the data presented here, by correcting for the number of genes analyses in the whole study. After applying this correction factor, a p-value of less than 0.00119 would be evidence of a strong significant association, a p-value of between 0.01 and 0.00120 would be evidence of a possible association.

RESULTS

We identified 3 polymorphisms within the gene for SLC6A4, 3 polymorphisms within the gene for HTR1B, 4 polymorphisms within the gene for HTR1D, 10 polymorphisms within the gene for HTR2A and 15 polymorphisms within the gene for HTR2C which had minor allele frequencies of greater than 0.10. The SNP identification number (HGVBASE at http://hgvbase.cgb.ki.se), the NCBI-34 mapping position, the region of the associated gene where the polymorphisms maps and the observed allele frequency in the control population are all shown in table 2 (available online).

Hardy-Weinberg equilibrium and pairwise linkage disequilibrium

One SNP was found to be out of HWE in the control population (rs2020934 in the gene encoding SLC6A4, HWE $p=4.67x10^{-9}$) and was excluded from further analysis; The strength of pairwise linkage disequilibrium (LD) varied across the 10 polymorphisms within the gene for HTR2A (Figure 1). Complete linkage disequilibrium across each of the loci for HTR1B, HTR1D (Brown et al. 2007) and HTR2C was observed in this control population (data not shown). There was no evidence of LD between the two remaining polymorphisms for SLC6A4.

Figure 1: Modular D' values for polymorphisms within the gene encoding HTR2A. There appears no discernable pattern of Linkage Disequilibrium(LD) across the gene for HTR2A with only polymorphisms rs582854 and rs2770293 appearing to be in strong LD.

Association of polymorphisms within the genes encoding, SLC6A4, HTR1B, HTR1D, HTR2A and HTR2C

SLC6A4

No evidence of association with DSM-IV™ sub-grouped AN or with AN as a whole, was found in this population with either of the polymorphisms (rs1872924 and rs3794809) identified in the serotonin transporter gene (SLC6A4) (Table 3 available online).

HRT1B

Both genotype (OR=0.53, 95%CI 0.37-0.78; p=0.0019) and allelic (OR=1.33, 95%CI 1.07-1.66; p=0.0103) frequencies at the polymorphism rs1213371 of HTR1B show significant association in a comparison between cases and controls (Table 4). There was no association with either rs1738538 or rs1145835 (Table 4, additional data of non-association available online).

Table 4: Results of case:control and subtype association analysis with HTR1B.

		Genotypic		Allelic		
Marker Comparison[a]	**Genotype[b]**	**Odds Ratio (95% CI)**	**p-Value**	**At risk Allele[b]**	**Odds Ratio (95% CI)**	**p-Value**
rs1213371						
Ca / Co	CC	0.53(0.37,0.78)	0.002	T	1.33(1.07,1.66)	0.010
RAN/ Co	CC	0.53(0.32,0.86)	0.029	T	1.39(1.04,1.87)	0.029
BPAN / Co	CC	0.55(0.32,0.94)	0.052	T	1.26(0.91,1.74)	0.160
RAN / BPAN	TT	0.84(0.42,1.67)	0.893	C	1.07(0.73,1.56)	0.773

[a] Ca = Cases (all anorexia nervosa cases); Co = Controls; RAN= Restricting anorexia nervosa cases; BPAN= Binge-purging anorexia nervosa cases

[b] "Genotype" does not represent the at risk genotype . Where the odds ratio is below 1 then the genotype is estimated to decrease risk; where the odds ratio is above 1 then the genotype is estimated to increase risk.

[c] The term "at risk allele" refers to the allele that occur more frequently in cases than in controls. In the case of the RAN/BPAN comparison, the RAN subjects are deemed 'cases' and BPAN subjects are deemed 'controls'.

HTR2A

Of the 10 SNPs analysed across the HTR2A gene, 2 were associated with AN. Polymorphism rs3742278 was found to be significantly associated at both the genotypic (OR=0.60, 95%CI 0.43-0.84; p=0.0003) and allelic (OR=1.65, 95%CI 1.23-2.22; p=0.0012) levels when comparing all cases with controls. This possible association appears to be specific to the BPAN with significant associations in both the genotypic (OR=0.47, 95%CI 0.29-0.75; p=0.0015) (however this fails to meet the stringent correction for significance at the p=0.00119 level) and allelic (OR=2.04, 95%CI 1.37-3.03; p=0.0006) comparisons with BPAN and controls which remains significant upon applying the correction for significance (figure 2).

Figure 2: Genotypic & allelic frequency and 95% CI for HTR2A polymorphism rs3742278. A significant difference was found in the allelic frequency between cases and controls (*p*=0.0011) there was a trend towards significant difference in the genotypic test, however this failed to stand up to the correction factor (p=0.0032). When comparing BPAN and controls the frequency of the G allele was significantly higher in the BPAN than in the controls (p= 0.0006) This association may to be a reflection of the extremely low frequency of the G,G genotype. However there were no significant differences when comparing the RAN with controls or with BPAN on either genotypic or allelic frequencies.

Polymorphism rs985934 was also significantly different in the control population from the case when comparing genotypic frequencies (OR=1.79, 95%CI 1.32-2.44; p=0.0007). This association appears to be driven by an association of RAN compared with controls in the genotypic level (OR=2.11, 95%CI 1.38-3.21; p=0.0021) (however this fails to meet the stringent correction for significance at the p=0.00119 level) (figure 3).

Figure 3: Genotypic & allelic frequency and 95% CI for HTR2A polymorphism rs985934. A significant difference was found in the genotypic frequency between cases and controls (*p*=0.0007). The frequency of the C,T genotype was increased in anorexia nervosa patients compared with the controls and this finding is complemented by a decreased frequency of T,T genotype in the anorexia nervosa population compared with the controls. In the comparison between RAN and controls there was a significant association (p=0.0021) in the genotypic analysis. There was no significant difference when comparing RAN with control by alleles (p=0.495). There was no significant differences either when comparing BPAN with control (Genotypic p=0.086, allelic p=0.079) there was also no significant differences between RAN and BPAN (genotypic p=0.355, allelic p=0.500)

Table 5: Results of case:control and subtype association analysis with HTR2A.

Marker Comparison[a]	Genotype[b]	Genotypic Odds Ratio (95% CI)	p-Value	At risk Allele[b]	Allelic Odds Ratio	p-Value
rs3742278						
Ca / Co	AA	0.60(0.43,0.84)	0.003	G	1.65(1.23,2.22)	0.001
RAN/ Co	AA	0.78(0.47,1.28)	0.487	G	1.28(0.82,2.00)	0.286
BPAN / Co	AA	0.47(0.29,0.75)	0.001	G	2.04(1.37,3.03)	0.001
RAN / BPAN	AA	0.49(0.27,0.87)	0.047	G	1.86(1.13,3.05)	0.018
rs985934						
Ca / Co	CT	1.79 (1.32,2.44)	0.001	C	1.21(0.97,1.51)	0.098
RAN/ Co	CT	2.11(1.38,3.21)	0.002	C	1.11(0.83,1.49)	0.495
BPAN / Co	TT	0.58(0.36,096)	0.086	C	1.34(0.97,1.86)	0.079
RAN / BPAN	CC	1.70(0.79,3.67)	0.355	C	1.14(0.78,1.66)	0.500

[a] Ca = Cases (all anorexia nervosa cases); Co = Controls; RAN= Restricting anorexia nervosa cases; BPAN= Binge-purging anorexia nervosa cases

[b] "Genotype" does not represent the at risk genotype . Where the odds ratio is below 1 then the genotype is estimated to decrease risk; where the odds ratio is above 1 then the genotype is estimated to increase risk.

[c] The term "at risk allele" refers to the allele that occur more frequently in cases than in controls. In the case of the RAN/BPAN comparison, the RAN subjects are deemed 'cases' and BPAN subjects are deemed 'controls'.

HTR2C

Of the 16 SNPs investigated within the gene for HTR2C, 10 were found to be significantly associated with BPAN when comparing genotypic frequencies with the control population however upon correction for multiple testing only 1 of these remained significant this was rs2428720 (OR=0.36, 95%CI 0.20-0.65; p=0.000276) (Table 6 full data on all non associated SNPS available online).

Table 6: Results of case:control and subtype association analysis with HTR2C.

		Genotypic			Allelic	
Marker Comparison[a]	**Genotype[b]**	**Odds Ratio (95% CI)**	**p-Value**	**At Risk Allele[b]**	**Odds Ratio**	**p-Value**
rs2428720						
Ca / Co	GG	0.61(0.43,0.86)	0.001	A	1.06(0.81,1.39)	0.729
RAN/ Co	GG	2.32(0.93,5.77)	0.201	G	1.16(0.81,1.66)	0.453
BPAN / Co	AG	0.36(0.20,0.65)	0.001	A	1.36(0.89,2.08)	0.151
RAN / BPAN	AG	0.47(0.24,0.91)	0.069	A	1.41(0.87,2.29)	0.179

[a] Ca = Cases (all anorexia nervosa cases); Co = Controls; RAN= Restricting anorexia nervosa cases; BPAN= Binge-purging anorexia nervosa cases

[b] "Genotype" does not represent the at risk genotype . Where the odds ratio is below 1 then the genotype is estimated to decrease risk; where the odds ratio is above 1 then the genotype is estimated to increase risk.

[c] The term "at risk allele" refers to the allele that occur more frequently in cases than in controls. In the case of the RAN/BPAN comparison, the RAN subjects are deemed 'cases' and BPAN subjects are deemed 'controls'.

Discussion

The outcome of this investigation has provided evidence for a substantial, and complex, interlinking of serotoninergic gene polymorphisms and genetic predisposition to subtypes of AN. The findings of the association analysis with HTR1D have previously been published (Brown et al. 2007). Significant associations have been demonstrated with genes for 4 separate serotoninergic receptors: genetic polymorphisms of the HTR1B and HTR1D genes appear to differentiate the RAN from control subjects; HTR2C alleles appear to differentiate BPAN from controls; and there is allelic variation in the HTR2A gene both within and between BPAN and RAN.

A considerable number of previous studies have used the candidate gene approach to ascertain the relationship between polymorphisms in the gene encoding HTR2A and anorexia nervosa. Most of these studies have reported no association but in the positive studies where DSM-1V classification had been applied, an association was reported to relate to the restricting subtype. In the present study, taking the anorexia nervosa group as a whole, a case-control comparison demonstrated a strong association ($p=0.0007$) with a polymorphism within the HTR2A gene. Moreover, whilst there was significant allelic variation between RAN and controls, there was also an association shown for BPAN and cases in comparison with controls. Consequently we have concluded that considerable allelic variation in polymorphisms of the HTR2A gene exists across the whole spectrum of patients with AN. However, the occurrence of differences between restricting and binge-purging anorexia nervosa suggests that specific features of this gene not only differ between all anorexia nervosa patients and controls but also differ between RAN and BPAN.

Of the 10 polymorphisms used to investigate the HTR2A gene, 8 did not demonstrate statistically significant associations. Polymorphism rs3742278 showed significant genotype and allelic variation between BPAN and controls (and between cases and controls), polymorphism rs985934 demonstrated highly significant genotypic association between cases and controls. Therefore, depending upon the specific polymorphism chosen in any candidate gene study, an association could be found with RAN, BPAN, both subtypes, or neither. This could account, at least in part, for the positive and negative findings of association previously reported in the literature (Klump and Gobrogge 2005). We are aware that diagnostic stability (or rigidity as it is sometimes called) is an issue and that diagnostic crossover is a feature in this disorder. An attempt to ensure this diagnostic stability was made by requiring subtype criteria to have been met for the previous 36 months. However there were some cases in which the eating disorder had not been diagnosed for the full 36 months and in these cases there would still be the possibility of diagnostic crossover prior to recruitment into the study. There would also be the possibility in those cases with diagnosis of longer than 36 months that diagnostic crossover had previously occurred. It is extremely difficult to eliminate the possibility of cross-over within the lifetime of the patient, but we feel that we have certainly eliminated the occurrence of cross-over within several years of the inception of this study.

A full genome linkage analysis study (Bergen et al. 2003) has, for the first time, implicated the HTR1D gene in AN. A region of human chromosome 1, incorporating this gene, was linked with genetic susceptibility to restricting, but not binge-purging, anorexia nervosa. We have recently provided a replication of these findings (Brown

et al. 2007). Of the 4 polymorphisms within this gene included in the analysis, one (rs856510) showed significant genotypic and allelic variation between RAN and controls, and a separate polymorphism (rs674386) also showed significant allelic variation between RAN and controls. These findings suggest that further analysis of the relationship between this gene and AN is warranted.

There is considerable evidence to implicate the HTR1B and HTR2C receptors in the control of appetite, and specifically with an inhibition of eating in animals and humans (Blundell and Halford 1998). There is therefore considerable circumstantial evidence for considering that variation in the genes for these 2 receptors could be associated with severe forms of human appetite disorders. There appears to have been no previous reports in the literature of association between AN and the HTR1B receptor gene. Of the 3 polymorphisms included in the current genotyping analysis, one (rs1213371) showed a significantly different frequency of occurrence in AN versus controls, and this was largely due to an association with the RAN subgroup. This polymorphism showed significant genotypic and allelic variation with the RAN subgroup. The association between polymorphisms within the gene for the HTR1B receptor (exclusively with the restricting subtype) is consistent with the known functional activity of this receptor in the appetite control systems. The HTR1B receptor agonist CP-94,253 inhibits food intake and intensifies satiety in rats (Halford and Blundell 1996) whilst in humans sumatriptan, which has a high affinity for the HTR1B (and HTR1D) receptor, has been shown to reduce intake at a meal (Boeler et al. 1997). Given this evidence, it is plausible to envisage that some adjustment to the structure of the HTR1B gene, if this caused a change in levels or sensitivity of HTR1B receptors, could promote a tendency to food restriction.

In contrast to the association of the HTR1B genes and RAN, a quite different pattern was observed for the HTR2C gene. Here, of the 16 polymorphisms investigated, 10 were found to be significantly and exclusively associated with the BPAN subtype. Moreover, there was also variation of the HTR2C genotypic and or allelic frequencies between the RAN and BPAN groups. Clearly, one reason for the significant associations with so many polymorphisms is because these polymorphisms were in LD indicating that a sizeable block of this gene is inherited as a complete unit. There is considerable evidence from animal studies that agonism at the HTR2C receptor is a sufficient condition for inhibition of food intake (Halford et al. 2004). Indeed, agonists at HTR2C receptors are currently in development as anti-obesity agents (Halford et al. 2004). This account is plausible since the HTR2C receptor agonist has been shown to decrease appetite and body weight in obese subjects (Smith et al. 2006).

In addition the transgenic HTR2C knock-out mouse displays hyperphagic behaviour and is obese (Tecott et al. 1995). A further interesting feature is that moderate food deprivation causes HTR2C receptor super-sensitivity (Cowen et al. 1996). Although three studies have found no association of the Cys-23-Ser alteration of the HTR2C receptor gene and either obese or underweight children (Hinney et al. 1997), anorexia nervosa patients (Nacmias et al. 1999), or sib-pair with anorexia nervosa (Karwautz et al. 2001) two other studies have reported an association between the Cys-23-Ser alteration of the HTR2C gene with low weight in teenage girls (Westberg et al. 2002) and minimum body mass index in AN (Hu et al. 2003). The results of the present study support an association but also go further to link the HTR2C receptor gene with

BPAN. In the light of the experimental evidence concerning the HTR2C receptor and appetite control, an association between polymorphisms within this gene and AN is entirely plausible. The reason why this gene should be associated exclusively with BPAN is not immediately clear. However there exists the interesting possibility that the powerful restriction of food intake in RAN and BPAN patients may be facilitated, at least in part, by 2 distinct serotoninergic receptor subtypes; the HTR1B in RAN and the HTR2C in BPAN. However, this suggestion should be treated with caution. The complex relationship between serotonin genes and the expression of appetite, and the reciprocal influence of dieting (and nutrition) on the sensitivity of serotonin receptors (as discussed by Hu et al. 2003) means that several possible neurochemical scenarios could be envisaged to mediate between polymorphisms of a gene for a specific receptor and the processes underlying the variability in symptomatology in AN patients. An area for further investigation is the link between tryptophan and serotonin and the possible mediating effects of tryptophan in eating disorders (Russo et al. 2007).

The outcome of this investigation supports the view that a complex variation in the genes from 4 different serotoninergic receptors (HTR1B, HTR1D, HTR2A and HTR2C) influences genetic susceptibility across the spectrum of symptomatology in AN. The investigation was driven by a strong hypothesis concerning the relationship between receptor sub-types in the serotoninergic systems and the clinical expression of sub-types of AN. We have decided to publish the results for all receptor sub-types together, rather than singly in order to provide the fullest picture of the inter-relationships. The study had considerable power and the associations (where significant) high probabilities. Some of the associations are complex. For example

with the HTR2A gene different polymorphisms show significant associations when comparisons are made between the whole group of AN cases, RAN or BPAN. This is intriguing and suggests that there is a controlling element in this gene relevant to the phenotypic expression of anorexia nervosa. For this, and the other associations seen for HTR1D, HTR1B and HTR2C genes further interpretation would be pure supposition. Taken as a whole these findings suggest an important relationship between serotonin receptor activity and clinical symptoms in AN.

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Financial Disclosure / Conflicts of Interest

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Online tables

Table 2: Details of SNPs used in genotyping of SLC6A4, HTR1B, HTR1D, HTR2A and HTR2

Gene Marker[a]	Chrm: Position[b]	Region	Flanking Region	Allele Frequency[c]
SLC6A4				
rs3794809	17:25555384	Intron	CTTTATGACT(A>G)TGAAATAATA	A: 0.453; G: 0.547
rs1872924	17:25570472	Intron	ATCATAGTCA(C>T)CTAGATTCCT	C: 0.209; T: 0.791
rs2020934	17:25585586	Intron	ACCGTTCCAA(T>C)ATGGATGAGT	C: 0.472; T: 0.528
HTR1B				
rs1738538	6:78224175	3' Flank	ATTTGGAGAC(C>T)CTCTGCCTGA	C: 0.865; T: 0.135
rs1145835	6:78225402	3' Flank	TTGCAGAATA(G>T)GAAAGCACAA	G: 0.069; T: 0.931
rs1213371	6:78236764	5' Flank	TGCAGCTGCA(C>T)CTTGGGAAAA	C: 0.582; T: 0.418
HTR1D				
rs604030	1:23263533	3' Flank	GGTCCCCAGA(G>T)GAACTGTGAG	G: 0.634; T: 0.366
rs652783	1:23259619	Intron	TTAATCATCA(T>C)GCTATCCTTA	C: 0.365; T: 0.635
rs674386	1:23267141	5' Flank	CATCAGGAAA(G>A)AAACCAAATT	A: 0.316; G: 0.684
rs856510	1:23269713	5' Flank	TGACAAGAAG(A>C)TACCATTTTC	A: 0.684; C: 0.316
HTR2A				
rs3803189	13:46306571	3' Flank	AAATAGCTAT(A>C)AATAGTGAAA	A: 0.860; C: 0.140
rs977003	13:46313002	Intron	TGGTGTAATT(T>G)AGTGCTTATT	G: 0.453; T: 0.547
rs3742278	13:46317578	Intron	AAGTGCACAC(G>A)TTGCTTATCA	A: 0.873; G: 0.127
rs643627	13:46326612	Intron	GAGCTCTATT(G>A)TGTGCCCCTC	A: 0.723; G: 0.277
rs1928042	13:46335217	Intron	TAAAGAGTCA(A>C)AATTGCAGTT	A: 0.763; C: 0.237
rs2770293	13:46336975	Intron	GAGTGAGATT(C>T)GTCTTTGCAA	C: 0.536; T: 0.464
rs582854	13:46343878	Intron	GCCATACTCA(G>T)CCAGTTAGGT	G: 0.538; T: 0.462
rs985934	13:46353726	Intron	AGAATACAAA(C>T)GGAAACTTGA	C: 0.386; T: 0.614
rs2025296	13:46361820	Intron	ATGATCTAAC(C>T)TGTTTGCTTC	C: 0.918; T: 0.082
rs2070040	13:46365627	Intron	ATCAGTATCA(G>A)CTGGAGAGCT	A: 0.452; G: 0.548
HTR2C				
rs475717	X:113638362	5' Flank	TATTTACCAC(A>C)GGACATAAAT	A: 0.832; C: 0.168
rs3795182	X:113639798	Intron	TCTCTTCCAT(A>G)TTTATAAATT	A: 0.829 ; G: 0.171
rs2376488	X:113651140	Intron	TTGGAGTTAA(A>C)TGACCTAAGT	A: 0.838; C: 0.162
rs2041675	X:113665758	Intron	TCTGAGTATA(C>T)TAAAAACCAC	C: 0.167; T: 0.833
rs2192371	X:113712819	Intron	TAATCTATTA(A>G)TCAAAATGTG	A: 0.653; G: 0.347
rs2069237	X:113742127	Intron	AAGTGCAAAA(A>G)CTTTTTTGGC	A: 0.165; G: 0.835
rs2248440	X:113784390	Intron	GTTATCTTTT(C>T)ACTAAAATAA	C: 0.832; T: 0.168
rs2428720	X:113796606	Intron	CTGATCTTAG(A>G)ACAAGTGCTT	A: 0.800; G: 0.200
rs2428712	X:113810464	Intron	CTTCTTTGCA(C>T)TTTCAGAGTG	C: 0.835; T: 0.165
rs2428707	X:113823339	Intron	GTAAAGCTAT(G>A)GTTCTAAAAC	A: 0.162; G: 0.838
rs2428698	X:113835653	Intron	GACATGGCAC(C>T)TCCAGTACAT	C: 0.836; T: 0.164
rs2428728	X:113847106	Intron	AACAATCAAG(G>A)TCACTTCTAA	A: 0.164; G: 0.836
rs2497501	X:113853775	Intron	TTCCTATACC(C>T)AACACTTAAT	C: 0.162; T: 0.838
rs1577456	X:113920551	Intron	CATGCTACTG(A>C)AAATGACAGG	A: 0.834; C: 0.166
rs1932268	X:113955110	Intron	GCCTGCCAGC(C>T)GGTGAGGACG	C: 0.165; T: 0.835
rs1414324	X:113971478	3' Flank	TTCAGCCAAA(A>G)TCAAATCACA	A: 0.169; G: 0.831

[a] NCBI v35 identifier, HGVBASE at http://hgvbase.cgb.ki.se, [b] According to NCBI v35 Map, [c] Allele frequencies from control samples

Table 3: Results of case:control and subtype association analysis with SLC6A4.

		Genotypic		Allelic		
Marker Comparison[a]	**Genotype[b]**	**Odds Ratio (95% CI)**	**p-Value**	**At risk Allele[b]**	**Odds Ratio (95% CI)**	**p-Value**
Rs3794809						
Ca / Co	AA	0.82(0.69,1.52)	0.994	G	1.01(0.81,1.27)	0.954
RAN/ Co	GG	0.68(0.42,1.01)	0.920	A	1.00(0.73,1.38)	1.000
BPAN / Co	AG	1.12(0.71,1.77)	0.854	G	1.03(0.75,1.43)	0.869
RAN / BPAN	AA	1.05(0.53,2.09)	1.000	A	1.03(0.70,1.53)	0.920
Rs1872924						
Ca / Co	CC	1.34(0.70,2.60)	0.644	C	1.11(0.86,1.44)	0.421
RAN/ Co	TT	0.76(0.35,1.04)	0.683	C	1.14(0.81,1.61)	0.475
BPAN / Co	CC	1.36(0.53,3.54)	0.827	C	1.08(0.73,1.61)	0.760
RAN / BPAN	CT	0.64(0.37,1.30)	0.593	T	1.20(0.76,1.87)	0.493

[a] Ca = Cases (all anorexia nervosa cases); Co = Controls; RAN= Restricting anorexia nervosa cases; BPAN= Binge-purging anorexia nervosa cases

[b] "Genotype" does not represent the at risk genotype . Where the odds ratio is below 1 then the genotype is estimated to decrease risk; where the odds ratio is above 1 then the genotype is estimated to increase risk.

[c] The term "at risk allele" refers to the allele that occur more frequently in cases than in controls. In the case of the RAN/BPAN comparison, the RAN subjects are deemed 'cases' and BPAN subjects are deemed 'controls'.

Table 4: Results of case:control and subtype association analysis with HTR1B.

		Genotypic		Allelic		
Marker Comparison[a]	**Genotype[b]**	**Odds Ratio (95% CI)**	**p-Value**	**At risk Allele[b]**	**Odds Ratio (95% CI)**	**p-Value**
rs1738538						
Ca / Co	CT	0.77(0.53,1.1.2)	0.308	C	1.18(0.85,1.64)	0.368
RAN/ Co	CT	0.61(0.36,1.05)	0.046	C	1.29(0.80,2.08)	0.296
BPAN / Co	TT	0.86(0.21,3.57)	1.000	C	1.08(0.68,1.70)	0.818
RAN / BPAN	CC	0.63(0.33,1.19)	0.322	T	1.48(0.83,2.65)	0.232
rs1145835						
Ca / Co	GG	0.63(0.03,12.5)	0.887	T	1.13(0.70,1.81)	0.638
RAN/ Co	TT	1.27(0.60,2.66)	0.583	T	1.25(0.60,2.58)	0.595
BPAN / Co	GG	0.61(0.03,12.5)	0.925	T	1.03(0.56,1.91)	1.000
RAN / BPAN	GT	1.62(0.68,3.88)	0.374	G	1.57(0.67,3.65)	0.390
rs1213371						
Ca / Co	CC	0.53(0.37,0.78)	0.002	T	1.33(1.07,1.66)	0.010
RAN/ Co	CC	0.53(0.32,0.86)	0.029	T	1.39(1.04,1.87)	0.029
BPAN / Co	CC	0.55(0.32,0.94)	0.052	T	1.26(0.91,1.74)	0.160
RAN / BPAN	TT	0.84(0.42,1.67)	0.893	C	1.07(0.73,1.56)	0.773

[a] Ca = Cases (all anorexia nervosa cases); Co = Controls; RAN= Restricting anorexia nervosa cases; BPAN= Binge-purging anorexia nervosa cases

[b] "Genotype" does not represent the at risk genotype . Where the odds ratio is below 1 then the genotype is estimated to decrease risk; where the odds ratio is above 1 then the genotype is estimated to increase risk.

[c] The term "at risk allele" refers to the allele that occur more frequently in cases than in controls. In the case of the RAN/BPAN comparison, the RAN subjects are deemed 'cases' and BPAN subjects are deemed 'controls'.

Table 5: Results of case:control and subtype association analysis with HTR2A.

Marker		Genotypic			Allelic	
Comparison[a]	Genotype[b]	Odds Ratio (95% CI)	p-Value	At risk Allele[b]	Odds Ratio	p-Value
rs3803189						
Ca / Co	AC	0.98(0.68,1.39)	0.981	A	1.02(0.74,1.39)	0.937
RAN/ Co	CC	2.40(0.58,9.84)	0.496	C	1.06(0.69,1.65)	0.822
BPAN / Co	CC	0.26(0.01,4.76)	0.621	A	1.10(0.70,1.71)	0.736
RAN / BPAN	CC	0.16(0.01,3.03)	0.169	C	1.06(0.62,1.83)	0.890
rs977003						
Ca / Co	GT	0.70(0.52,0.95)	0.069	T	1.08(0.87,1.34)	0.507
RAN/ Co	GT	0.73(0.48,1.11)	0.325	T	1.02(0.76,1.37)	0.940
BPAN / Co	GT	0.68(0.43,1.06)	0.180	T	1.16(0.84,1.59)	0.417
RAN / BPAN	TT	1.07(0.62,1.86)	0.969	T	1.06(0.73,1.54)	0.773
rs3742278						
Ca / Co	AA	0.60(0.43,0.84)	0.003	G	1.65(1.23,2.22)	0.001
RAN/ Co	AA	0.78(0.47,1.28)	0.487	G	1.28(0.82,2.00)	0.286
BPAN / Co	AA	0.47(0.29,0.75)	0.001	G	2.04(1.37,3.03)	0.001
RAN / BPAN	AA	0.49(0.27,0.87)	0.047	G	1.86(1.13,3.05)	0.018
rs643627						
Ca / Co	GG	0.62(0.33,1.16)	0.254	A	1.09(0.85,1.39)	0.497
RAN/ Co	AG	1.26(0.82,1.93)	0.515	G	1.02(0.74,1.41)	0.933
BPAN / Co	GG	0.44(0.14,1.36)	0.294	A	1.23(0.85,1.78)	0.271
RAN / BPAN	GG	0.41(0.12,1.43)	0.343	A	1.24(0.81,1.91)	0.328
rs1928042						
Ca / Co	AA	1.15 (0.83,1.58)	0.730	A	1.11(0.85,1.45)	0.457
RAN/ Co	AA	1.50(0.96,2.37)	0.214	A	1.41(0.95,2.08)	0.090
BPAN / Co	AA	0.84(0.53,1.35)	0.736	C	1.16(0.80,1.68)	0.495
RAN / BPAN	AA	0.52(0.30,0.93)	0.048	C	1.81(1.12,2.91)	0.015
rs2770293						
Ca / Co	CC	1.07 (0.76,1.51)	0.938	C	1.03(0.83,1.29)	0.776
RAN/ Co	TT	0.74(0.42,1.27)	0.449	C	1.09(0.80,1.49)	0.579
BPAN / Co	TT	0.71(0.45,1.12)	0.298	T	1.03(0.75,1.42)	0.871
RAN / BPAN	TT	0.68(0.39,1.18)	0.354	T	1.03(0.70,1.52)	0.921
rs582854						
Ca / Co	TT	0.85(0.57,1.23)	0.634	G	1.11(0.89,1.37)	0.379
RAN/ Co	TT	0.69(0.40,0.69)	0.316	G	1.13(0.84,1.52)	0.453
BPAN / Co	TT	0.79(0.51,1.23)	0.536	G	1.08(0.79,1.48)	0.686
RAN / BPAN	TT	0.75(0.44,1.26)	0.566	G	1.00(0.69,1.46)	1.000
rs985934						
Ca / Co	CT	1.79 (1.32,2.44)	0.001	C	1.21(0.97,1.51)	0.098
RAN/ Co	CT	2.11(1.38,3.21)	0.002	C	1.11(0.83,1.49)	0.495
BPAN / Co	TT	0.58(0.36,096)	0.086	C	1.34(0.97,1.86)	0.079
RAN /	CC	1.70(0.79,3.67)	0.355	C	1.14(0.78,1.66)	0.500

Marker		Genotypic			Allelic	
Comparison[a]	Genotype[b]	Odds Ratio (95% CI)	p-Value	At risk Allele[b]	Odds Ratio	p-Value
BPAN rs2025296						
Ca / Co	CT	1.69 (1.15,2.50)	0.025	T	1.45(1.01,2.07)	0.051
RAN/ Co	CT	1.71(1.02,2.86)	0.115	T	1.56(0.98,2.50)	0.071
BPAN / Co	CT	1.68(0.93,3.03)	0.152	T	1.32(0.77,2.28)	0.377
RAN / BPAN	TT	0.40(0.02,10.00)	0.858	C	1.22(0.67,2.22)	0.544
rs2070040						
Ca / Co	GG	1.35 (0.98,1.88)	0.196	G	1.18(0.95,1.48)	0.142
RAN/ Co	AA	0.61(0.33,1.12)	0.159	G	1.34(0.98,1.83)	0.072
BPAN / Co	AG	0.67(0.42,1.05)	0.203	G	1.03(0.75,1.42)	0.870
RAN / BPAN	AA	2.29(1.13,4.67)	0.058	A	1.36(0.92,2.01)	0.138

[a] Ca = Cases (all anorexia nervosa cases); Co = Controls; RAN= Restricting anorexia nervosa cases; BPAN= Binge-purging anorexia nervosa cases

[b] "Genotype" does not represent the at risk genotype . Where the odds ratio is below 1 then the genotype is estimated to decrease risk; where the odds ratio is above 1 then the genotype is estimated to increase risk.

[c] The term "at risk allele" refers to the allele that occur more frequently in cases than in controls. In the case of the RAN/BPAN comparison, the RAN subjects are deemed 'cases' and BPAN subjects are deemed 'controls'.

Table 6: Results of case:control and subtype association analysis with HTR2C.

		Genotypic			Allelic	
Marker Comparison[a]	**Genotype[b]**	**Odds Ratio (95% CI)**	**p-Value**	**At Risk Allele[b]**	**Odds Ratio**	**p-Value**
rs475717						
Ca / Co	CC	1.65(0.79,3.43)	0.317	C	1.02(0.76,1.36)	0.941
RAN/ Co	AA	0.81(0.78,1.26)	0.618	C	1.21(0.83,1.77)	0.369
BPAN / Co	AC	0.53(0.30,0.93)	0.036	A	1.21(0.78,1.88)	0.442
RAN / BPAN	AC	0.48(0.25,0.91)	0.068	A	1.43(0.86,2.37)	0.201
rs3795182						
Ca / Co	GG	0.40(0.13,1.25)	0.194	A	1.12(0.82,1.53)	0.486
RAN/ Co	GG	0.26(0.05,1.41)	0.078	A	1.68(1.06,2.68)	0.025
BPAN / Co	AG	1.60(0.98,2.64)	0.183	G	1.32(0.87,2.02)	0.216
RAN / BPAN	AA	0.49(0.26,0.91)	0.047	G	1.84(1.06,3.20)	0.036
rs2376488						
Ca / Co	CC	2.03(0.95,4.33)	0.104	C	1.01(0.75,1.35)	1.000
RAN/ Co	CC	2.03(0.68,6.06)	0.482	C	1.19(0.80,1.76)	0.409
BPAN / Co	AC	0.54(0.30,0.96)	0.050	A	1.20(0.77,1.87)	0.441
RAN / BPAN	AC	0.53(0.28,1.03)	0.163	A	1.33(0.80,2.23)	0.297
rs2041675						
Ca / Co	CC	1.94(0.91,4.12)	0.136	C	1.02(0.76,1.36)	0.941
RAN/ Co	CC	1.65(0.57,4.72)	0.578	C	1.18(0.80,1.72)	0.425
BPAN / Co	CT	0.53(0.30,0.95)	0.031	T	1.17(0.75,1.83)	0.507
RAN / BPAN	CT	0.5(0.26,0.96)	0.096	T	1.37(0.82,2.28)	0.247
rs2192371						
Ca / Co	AG	1.09(0.80,1.47)	0.832	A	1.01(0.81,1.27)	0.954
RAN/ Co	GG	0.55(0.27,1.15)	0.194	A	1.13(0.83,1.54)	0.435
BPAN / Co	AA	1.39(0.75,2.59)	0.579	G	1.13(0.81,1.57)	0.495
RAN / BPAN	AA	2.43(1.05,5.62)	0.070	G	1.20(0.81,1.78)	0.368
rs2069237						
Ca / Co	AA	1.86(0.90,3.81)	0.134	A	1.01(0.76,1.35)	1.000
RAN/ Co	AA	1.86(0.68,5.07)	0.412	A	1.25(0.86,1.83)	0.272
BPAN / Co	AG	0.47(0.26,0.85)	0.020	G	1.31(0.83,2.07)	0.268
RAN / BPAN	AG	0.45(0.23,0.88)	0.057	G	1.51(0.90,2.52)	0.123
rs2248440						
Ca / Co	TT	2.23(1.09,4.59)	0.039	T	1.02(0.77,1.36)	0.883
RAN/ Co	TT	2.07(0.75,5.74)	0.369	T	1.22(0.84,1.78)	0.322
BPAN / Co	CT	0.45(0.25,0.82)	0.007	C	1.22(0.78,1.90)	0.382
RAN / BPAN	CT	0.45(0.23,0.88)	0.051	C	1.39(0.84,2.30)	0.205
rs2428720						
Ca / Co	GG	0.61(0.43,0.86)	0.001	A	1.06(0.81,1.39)	0.729
RAN/ Co	GG	2.32(0.93,5.77)	0.201	G	1.16(0.81,1.66)	0.453
BPAN / Co	AG	0.36(0.20,0.65)	0.001	A	1.36(0.89,2.08)	0.151
RAN / BPAN	AG	0.47(0.24,0.91)	0.069	A	1.41(0.87,2.29)	0.179
rs2428712						

Marker Comparison[a]	Genotype[b]	Genotypic Odds Ratio (95% CI)	p-Value	At Risk Allele[b]	Allelic Odds Ratio	p-Value
Ca / Co	TT	2.09(1.00,4.36)	0.705	T	1.03(0.77,1.83)	0.880
RAN/ Co	TT	1.79(0.61,5.22)	0.466	T	1.23(0.83,1.82)	0.351
BPAN / Co	CT	0.47(0.26,0.85)	0.009	C	1.20(0.77,1.87)	0.441
RAN / BPAN	CT	0.44(0.23,0.87)	0.048	C	1.37(0.82,2.29)	0.243
rs2428707						
Ca / Co	AA	2.13(0.99,4.60)	0.059	G	1.01(0.76,1.36)	0.940
RAN/ Co	AA	1.73(0.59,5.04)	0.559	A	1.18(0.81,1.73)	0.422
BPAN / Co	AG	0.43(0.23,0.79)	0.004	G	1.29(0.81,2.05)	0.310
RAN / BPAN	AG	0.45(0.22,0.85)	0.040	G	1.46(0.87,2.46)	0.190
rs2428698						
Ca / Co	CT	0.70(0.49,1.02)	0.089	C	1.13(0.83,1.53)	0.448
RAN/ Co	TT	0.96(0.60,1.49)	0.975	C	1.03(0.69,1.53)	0.919
BPAN / Co	TT	0.45(0.25,0.84)	0.010	C	1.26(0.79,2.00)	0.364
RAN / BPAN	CT	0.47(0.24,0.94)	0.048	C	1.22(0.71,2.09)	0.495
rs2428728						
Ca / Co	AA	2.19(1.04,460)	0.048	A	1.01(0.75,1.35)	1.000
RAN/ Co	AA	1.74(0.60,5.08)	0.574	A	1.16(0.79,1.70)	0.485
BPAN / Co	GG	0.46(0.25,0.83)	0.005	G	1.18(0.76,1.84)	0.507
RAN / BPAN	AG	0.45(0.23,0.88)	0.046	G	1.32(0.79,2.19)	0.305
rs2497501						
Ca / Co	TT	0.71(0.50,1.03)	0.054	T	1.05(0.78,1.41)	0.764
RAN/ Co	CC	1.44(0.46,4.51)	0.865	C	1.05(0.76,1.56)	0.839
BPAN / Co	TT	0.47(0.26,0.85)	0.007	T	1.17(0.75,1.82)	0.506
RAN / BPAN	CT	0.50(0.25,0.98)	0.074	T	1.18(0.70,1.98)	0.597
rs1577456						
Ca / Co	CC	2.19(1.04,4.59)	0.043	C	1.00(0.75,1.33)	1.000
RAN/ Co	CC	1.75(0.60,5.10)	0.545	C	1.18(0.80,1.72)	0.424
BPAN / Co	AC	0.45(0.26,0.81)	0.004	A	1.21(0.78,1.88)	0.389
RAN / BPAN	AC	0.47(0.25,0.91)	0.056	A	1.31(0.79,2.17)	0.309
rs1932268						
Ca / Co	CC	2.19(1.04,4.59)	0.042	C	1.00(0.75,1.34)	1.000
RAN/ Co	CC	1.74(0.60,5.08)	0.545	C	1.18(0.81,1.73)	0.423
BPAN / Co	CT	0.43(0.24,0.79)	0.003	T	1.23(0.79,1.91)	0.382
RAN / BPAN	CT	0.43(0.23,0.88)	0.046	T	1.32(0.79,2.19)	0.305
rs1414324						
Ca / Co	AA	2.23(1.12,5.07)	0.055	A	1.09(0.81,1.46)	0.593
RAN/ Co	GG	0.70(0.44,1.11)	0.212	A	1.41(0.95,2.10)	0.110
BPAN / Co	AG	0.43(0.23,0.78)	0.003	G	1.24(0.79,1.93)	0.379
RAN / BPAN	AG	0.40(0.20,0.79)	0.022	G	1.47(0.88,2.47)	0.152

[a] Ca = Cases (all anorexia nervosa cases); Co = Controls; RAN= Restricting anorexia nervosa cases; BPAN= Binge-purging anorexia nervosa cases

[b] "Genotype" does not represent the at risk genotype . Where the odds ratio is below 1 then the genotype is estimated to decrease risk; where the odds ratio is above 1 then the genotype is estimated to increase risk.
[c] The term "at risk allele" refers to the allele that occur more frequently in cases than in controls. In the case of the RAN/BPAN comparison, the RAN subjects are deemed 'cases' and BPAN subjects are deemed 'controls'.