Association Study of the Estrogen Receptor I Gene (ESR1) in Anorexia Nervosa and Eating Disorders: No Replication Found

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ABSTRACT
Objective: The female preponderance and onset around puberty in the majority of eating disorders (EDs) suggest that sex hormones, like estrogens, may be involved in the onset of these disorders. An eight-SNP haplotype at the estrogen receptor I (ESR1) gene was found to be associated with anorexia nervosa (AN) (Versini et al., Neuropsychopharmacology, 35, 1818–1825, 2010) and three SNPs from this haplotype (rs726281, rs2295193, and rs3798577) were associated with AN and/or EDs. Our objective was to replicate these findings in an independent cohort of 520 patients with an eating disorder, of whom 244 had AN (restricting type) from the GenED study and 2,810 random women from the Netherlands Twin Registry.

Method: The frequencies of the eight-SNP haplotype and three ESR1 SNPs were compared between patients with an eating disorder, with AN (restricting type), with bulimia nervosa (BN), and the control women.

Results: Neither the haplotype nor the three ESR1 SNPs were associated with EDs, BN, AN, or restricting type AN.

Discussion: Despite sufficient statistical power, the associations reported by Versini et al. (Neuropsychopharmacology, 35, 1818–1825, 2010) were not replicated.

Keywords: estrogen; eating disorders; anorexia nervosa; genetic association; ESR1

Introduction
The clear sex difference in eating disorders (EDs),1 together with the age of onset around puberty for most EDs,2,3 indicates that sex hormones, like estrogens, might be involved in the onset of EDs. Estradiol activates estrogen receptors α and β, regulate transcriptional activation,4 and also appear to be involved in the regulation (inhibition) of food intake.5,6 The estrogen receptor I (ESR1) and ESR2 genes, which respectively encode estrogen receptor α and β, are expressed in several tissues throughout the body including the brain.4

Several ESR1 and ESR2 polymorphisms have been tested for association with EDs.7–10 Rosenkranz et al.8 did not find an association between ESR2 and anorexia (AN) or bulimia nervosa (BN), whereas two other studies did find associations between common ESR2 polymorphisms and AN9 or BN.10 Recently, a large family-based association study examined common genetic variation at the ESR1 and ESR2 genes in relation to AN.7 A haplotype of eight ESR1 SNPs was associated with AN restricting type (OR = 3.1, p < 6 × 10⁻⁶). In addition, three SNPs from this haplotype were associated with AN and/or EDs. A significant over-transmission was detected between AN and ESR1 rs726281 (OR = 1.5, p < .02) and rs2295193 (OR = 1.4, p < .02). In addition, rs3798577 was associated with restricting type AN (OR = 1.6, p < .02) and with EDs in a population-based sample of 693 women (p < .008).

Because replication is essential to identify robust genetic associations, the current study tried to replicate the findings from this previous study.7 The haplotype of the eight ESR1 SNPs and the three separate ESR1 SNPs were tested for association in...
520 patients with an ED, of whom 244 patients had AN (142 restricting type) and 143 had BN from the GenED study, and 2,810 random control women from the Netherlands Twin Registry (NTR).

**Method**

**Participants**

Five hundred twenty female patients with a DSM-IV ED diagnosis participated in the GenED study.\(^{11}\) This study was approved by the committee for mental health institutions in the Netherlands (METiGG). All participants gave written informed consent. The majority of patients \((n = 244)\) had AN (142 restricting, 102 binge-eating/purging type), 143 had BN, and 133 patients had an ED not otherwise specified. Eighty-three percent of the patients with AN, 76\% of the patients with BN, and 95\% of the patients with an ED not otherwise specified had a current ED diagnoses, the remaining patients had a lifetime diagnoses.

The NTR collects data on multiples (twins or triplets) and their family members in longitudinal survey studies.\(^{12,13}\) Subgroups have provided DNA samples. Data and DNA collection were approved by the ethics committee of the VU University. In the current study, 2,810 unrelated women, not screened for EDs, were drawn from the NTR. For 365 women direct ESRI genotyping was performed. For the remaining 2,445 women imputed ESRI genotype data from previous GWA studies\(^{12,13}\) were available. Both control and patient groups were of European descent.

**Genotype Measurements**

Eight ESRI SNPs (rs726281, rs3020407, rs17081994, rs2982712, rs3020371, rs2228480, rs3798577, rs2295193) were genotyped by mass spectrometry (the homogeneous MassARRAY system; Sequenom, San Diego, CA) using standard conditions in the patients and 365 control women.

In the majority of the control women, SNPs were imputed by SNPtest (version 2, merge release 5). As a quality control measure the \(r^2\) for the imputation was calculated, the \(r^2\) ranged between .97 and .99 for all SNPs, except for rs726281 and rs3020407 for which an \(r^2\) of .93 and .89 was found respectively. As an additional quality control step, the correlation between the genotyped and imputed SNPs was calculated in the 365 control women. The correlations for rs726281 and rs3020407 were moderate, .83 and .66 respectively, for the remaining SNPs the correlations ranged between .93 and .98. The lower \(r^2\) and correlations indicate that the quality of the imputation for rs726281 and rs3020407 was insufficient, and therefore only the directly genotyped data for these two SNPs were used. For the remaining six SNPs, the imputed genotypes were used. SNPtest calculates dosage data (ranging from 0 to 2) based on the
probabilities of the three possible genotypes for each SNP. To take into account the uncertainty of the imputed genotype we applied the following thresholds on the dosage data: 0–0.10 for homozygotes of the common allele, 0.90–1.10 for heterozygotes, 1.90–2 for homozygotes of the minor allele.

Statistical Analyses

The \( \chi^2 \) test for Hardy–Weinberg equilibrium (HWE) was calculated in the controls using the HWE program of LINKUTIL (http://linkage.rockefeller.edu/ott/linkutil.htm). Power calculations were performed in Quanto version 1.2.4 (2009).

The haplotype frequency of the eight-SNP ESR1 haplotype was compared in patients with an ED, patients with AN, patients with restricting type AN, patients with BN, and the control group, using Haploview (version 4.2). Furthermore, the allele frequencies of three SNPs (rs726281, rs2295193, and rs3798577) were compared between patient and control groups using Pearson's chi-square test (IBM-SPSS version 19).

Results

In the control group none of the SNPs revealed a departure from HWE \( (p > 0.05) \). This study had adequate power (85% power at \( \alpha \)-level 0.05, log-additive/allelic model) to detect effect sizes that were reported by Versini et al. We had statistical power to detect effect sizes of at least 1.23 for EDs, between 1.33 and 1.45 for AN, and between 1.45 and 1.55 for restricting type AN and BN.

The results of the association analyses for the haplotype and the three single SNPs are shown in Table 1. There was no significant difference in the frequency of the eight-SNP ESR1 haplotype (rs726281*A-rs3020407*A-rs17081994*T-rs2982712*T-rs3020371*C-rs2228480*G-rs3798577*T-rs2295193*C) which was overtransmitted in restricting type AN in the Versini study, between controls (frequency 0.22) and patients with either an ED (frequency 0.21), BN (frequency 0.21), AN (frequency 0.22), or restricting type AN (frequency 0.25). There were no significant differences in minor allele frequency for rs726281, rs3798577, and rs2295193 between control women and patients with an ED, BN, AN, or restricting type AN.

Discussion

This study aimed to replicate findings from a family-based association study that reported associations between AN and an eight-SNP ESR1 haplotype, ESR1 rs726281 and rs2295193, and an association between rs3798577 and EDs. Although the haplotype had a comparable frequency in our sample, it was not associated with either EDs, BN, AN, or restricting type AN. In addition, the frequency of none of the three ESR1 SNPs differed between the groups. Altogether, we could not confirm the previous findings in the current study.

We had adequate statistical power to detect an effect ranging from 1.23 to 1.33 for ED, from 1.33 to 1.45 for AN, and from 1.45 to 1.55 for restricting type AN and BN. Given the ORs reported in the Versini study, the power in the current study should have been sufficient to detect these effects. As there is a general tendency for initial studies to overestimate effect sizes, it is possible that associations were missed in the current study because of insufficient power to detect smaller effects. This could especially be the case for ESR1 rs726281, for which a smaller number of control women were available due to the poorer quality of the imputation.

In the present study, the control groups were not screened for the absence or presence of an ED. Lifetime prevalence in women ranges between 0.9 and 3.5 for different types of EDs. Because of this low prevalence only a small number of women will be affected in the population, of the total 2,810 controls approximately 165 could be affected. Additional analyses, not reported here, show that taking these possibly affected controls into consideration would not change our results.

From a biological perspective, the influence of estrogens on the development of EDs is plausible. There is some preliminary evidence that estradiol is a moderator of genetic effects on disordered eating during puberty. Although it is still unclear whether estrogens are involved and what role they might play in EDs. The current study failed to replicate previously reported associations between the ESR1 gene and AN. To determine with certainty whether the ESR1 gene is involved in the development of EDs larger future studies are required.

References

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