



## **Estrogen receptor 1 gene (ESR1) is associated to restrictive anorexia nervosa**

Audrey Versini, Nicolas Ramoz, Yann Le Strat, Susann Friedel, Stefan Ehrlich, Claudette Boni, Anke Hinney, Johannes Hebebrand, Lucia Romo, Julien-Daniel Guelfi, et al.

### **► To cite this version:**

Audrey Versini, Nicolas Ramoz, Yann Le Strat, Susann Friedel, Stefan Ehrlich, et al.. Estrogen receptor 1 gene (ESR1) is associated to restrictive anorexia nervosa. *Neuropsychopharmacology*, 2010, 35 (8), pp.1818-25. <10.1038/npp.2010.49>. <hal-00524128>

**HAL Id: hal-00524128**

**<https://hal.science/hal-00524128v1>**

Submitted on 7 Oct 2010

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

**TITLE PAGE**

**Estrogen receptor 1 gene (ESR1) is associated to restrictive anorexia nervosa**

Audrey Versini<sup>1\*</sup>, Nicolas Ramoz<sup>1\*</sup>, Yann Le Strat<sup>1</sup>, Susann Friedel<sup>2</sup>, Stefan Ehrlich<sup>3</sup>, Claudette Boni<sup>1</sup>, Anke Hinney<sup>2</sup>, Johannes Hebebrand<sup>2</sup>, Lucia Romo<sup>4</sup>, Julien-Daniel Guelfi<sup>5</sup>, Philip Gorwood<sup>1,5</sup>

<sup>1</sup> INSERM U894-Team 1, Center of Psychiatry and Neurosciences, University Paris Descartes, 2ter rue d'Alésia, 75014 Paris, France.

<sup>2</sup> Department of Child and Adolescent Psychiatry, University of Duisburg-Essen, Virchowstr. 174, 45147 Essen, Germany.

<sup>3</sup> Department of Child and Adolescent Psychiatry, Psychosomatic Medicine and Psychotherapy, Charité - Universitätsmedizin Berlin, Campus Virchow-Klinikum, Berlin, Germany.

<sup>4</sup> AP-HP. Psychiatry, Louis Mourier Hospital, 178 Rue des Renouillers, 92700 Colombes, France.

<sup>5</sup> Sainte-Anne Hospital Center (CHSA), Clinic for Mental and Brain Diseases (CMME), 100 rue de la Santé, 75014 Paris, France.

\*These authors contributed equally to the work

**Running title:** ESR1 and Restrictive Anorexia Nervosa

**Correspondence to:**

Nicolas Ramoz

INSERM U894-Team 1, Center of Psychiatry and Neurosciences, 2 ter rue d'Alésia, 75014

Paris, France.

Phone: (33) 1-40-78-92-83

Fax: (33) 1-40-78-92-80

Email: [nicolas.ramoz@inserm.fr](mailto:nicolas.ramoz@inserm.fr)

## ABSTRACT

Anorexia nervosa is a highly heritable young-onset psychiatric illness which aetiology remains unknown. Estrogen alpha- and beta-receptors, encoded by *ESR1* and *ESR2* genes, are involved in food intake regulation and eating behaviour, and may have a potential role in AN. We performed a family-based association study of 17 single-nucleotide polymorphisms (SNPs) encompassing *ESR1* and *ESR2* genes in a cohort of 321 French AN families. We attempted to replicate this finding in a cohort of 41 RAN families and in a population-based study of 693 young women.

Using the transmission disequilibrium test, a significant over-transmission was detected between AN and *ESR1* rs726281 and rs2295193. These SNPs and another among *ESR1* were more specifically associated with the restrictive AN subtype (RAN) (rs726281,  $p=0.005$ , odds ratio [OR]=2.1, 95% Confidence Interval [95%CI]=1.2-3.6; rs3798577,  $p=0.021$ , OR=1.6, 95%CI=1.1-2.3; and rs2295193,  $p=0.007$ , OR=1.7, 95%CI=1.2-2.5). A large 8-SNPs haplotype of *ESR1* gene was also associated with AN ( $p<0.0001$ , OR=3.1, 95%CI=1.8-5.1). Association of *ESR1* SNPs and RAN was driven by paternal over-transmissions ( $p<0.0001$ , OR=3.7, 95%CI=1.9-7.3). Furthermore, we confirmed the preferential paternal over-transmission of the *ESR1* rs726281 on the independent German sample of 41 RAN trios ( $p=0.025$ , OR=3, 95%CI=1.1-8.3). Lastly, rs3798577 was associated with eating disorders in a population-based sample of 693 women ( $p<0.01$ ).

Our findings are strongly in favour of an association between *ESR1* polymorphisms and anorexia nervosa. Particularly, *ESR1* gene confers a high risk of vulnerability to the restrictive subtype of anorexia nervosa, and suggests that the estrogen pathway has to be further investigated in AN.

**KEYWORDS**

Anorexia nervosa; restrictive type; binge-eating/purging type; estrogen receptors;  
transmission disequilibrium test; population-based sample

## INTRODUCTION

Anorexia nervosa (AN, MIM 606788) is an eating disorder characterized by weight loss (body mass index  $< 17.5 \text{ kg/m}^2$ ) or failure to make expected weight gain during period of growth, intense fear of gaining weight or becoming fat, body image disturbance and amenorrhea (American Psychiatric Association, 1994; Gorwood, *et al* 2003; Ramoz, *et al* 2007). The prevalence of AN is between 0.3 to 0.6% (Hudson, *et al* 2007; Ramoz, *et al* 2007), with one of the highest mortality rate (about 10% per decade) mainly due to cachexia and suicide (Harris and Barraclough, 1994; Hoek, 2006). Furthermore, the heritability of AN is high (70%), thus increasing the potential interests of genetic approaches (Gorwood, *et al* 2003; Ramoz, *et al* 2007). Studies have found evidence of a high genetic risk for AN, especially in the restrictive AN subgroup probably because of heterogeneity reduction of the phenotype, with, therefore, an increased power to detect an association (Grice, *et al* 2002; Gershon, *et al* 1984).

According to the DSM-IV, AN patients are subdivided in two categories: restrictive type (RAN), characterizing patients with restricted food intake without binge eating or purging episodes, and binge eating/purging type (BPAN), characterizing patients with binge eating/purging episodes during anorexia and bulimia phases (American Psychiatric Association, 1994). RAN and BPAN patients represent two subtypes with specific clinical features, including significant lower BMI in RAN than in BPAN, and an increased impulsivity and a higher rate of self-harm and suicide in BPAN than RAN (Eddy, *et al* 2002; Foulon, *et al* 2007; Milos, *et al* 2004). Hormone concentrations may also help to distinguish these two subtypes, since leptin is decreased in RAN compared to BPAN (Eddy, *et al* 2002).

Biological pathways of sexual hormones were poorly investigated in AN, although the involvement of the estrogenic pathway in AN is supported by several cues. First a female predominance is observed in patients with a ratio of 9 women to 1-3 men (Hudson, *et al* 2007; Ramoz, *et al* 2007). Second, the onset of AN around puberty is correlated to the presence of estrogenic peaks. ). Third, recent studies proposed that sex hormones have a role in the risk of eating disorders (Culbert KM, *et al* 2008; Procopio, *et al* 2007) but this observation was not replicated by other authors (Raevuori, *et al* 2008; Baker, *et al* 2009). Fourth, the anorexic effect of high estrogen levels was found in animal models (Couse and Korak, 1999; Wade and Gray, 1979). Moreover, estradiol levels were lower in anorexia patients than in controls (Ohwada, *et al* 2007; Brambilla, *et al* 2003). Fifth, the *ESR1* and *ESR2* genes, which code for estrogen alpha and beta receptors and are expressed in non-overlapping brain regions (Osterlund, *et al* 2000; Osterlund and Hurd, 2001), colocalize with corticotrophin releasing factor and modulate its expression (Dagnault and Richard, 1997; Bao, *et al* 2005). They participate in the regulation of the hypothalamic pituitary adrenal (HPA) axis (Licinio, *et al* 1996). Thus, the disruption of the HPA axis reported in AN might be due to an alteration in the estrogen pathway in patient (Van de Stolpe, *et al* 2004). Finally, the involvement of the estrogen alpha receptor in the regulation of food intake and eating behaviour was confirmed in mouse models (Musatov, *et al* 2006; Musatov, *et al* 2007). Two case-control studies of AN with *ESR1* and *ESR2* genes were previously published (Eastwood, *et al* 2002; Rosenkranz, *et al* 1998). They reported no association of the *ESR1* gene with AN, and an inconsistent association of AN with different variants within *ESR2* gene.

Thus, our hypothesis is to identify an association between estrogen receptor and anorexia nervosa, especially in RAN subgroup, because a diminution of estradiol level was

more important in the restrictive compared to the bingeing-purging subtype (Ohwada, *et al* 2007). We more precisely expect to find an association between anorexia nervosa and *ESR1* gene because ovariectomized rat showed an implication of ESR1 but not of ESR2 receptor in food intake, body weight and meal size (Santonello, *et al* 2007). Finally, we have been also analysing the role of parental imprinting of this estrogen receptor in anorexia nervosa, because ESR1 belongs to the 6q25 region which could be subjected to parental imprinting according to database (Nikawa, *et al* 1996; Morison, *et al* 2005). Furthermore, the absence of ESR1 transcription could be a result of aberrant methylation of promoter CpG islands, as shown for example in breast cancers (Wilson, *et al* 2008).

Here, we performed the first family-based association study on a large number of AN families of French origin looking for an association between *ESR1* gene and AN, and we took advantage of the narrowing definition of the disorder to show that this association is driven by the restrictive subtype of AN. We attempted to replicate the family-based association in an independent sample of German families (replication of the TDT approach), as well as in an independent population-based sample recruited from the general population.



## MATERIALS AND METHODS

### *Subjects and Phenotype*

The study was approved by each national ethics committee. All participants (and if underage, their parents) gave written informed consent.

The first family-based cohort was composed of 321 families of French origin, including 210 complete trios, of whom 102 were described previously (Gorwood, *et al* 2003). All participants were assessed by clinicians using the face-to-face semi-structured Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger, *et al* 1994). Diagnosis of AN were made according to DSM-IV criteria (American Psychiatric Association, 1994), and included AN restrictive type (RAN) with no lifetime binge-eating/purging episode (N=153 families), and AN binge-eating/purging type (BPAN) with at least one lifetime bingeing/purging episode, during anorexia or bulimia phases (N=154 families). Information for subtype was incomplete for 14 probands.

Furthermore, the Eating Disorder Inventory (EDI-2) (Garner, *et al* 1983) and the Eating Attitude Test (EAT-26) (Garner, *et al* 1982) were used to measure the psychological and behavioural dimensions and the broad range of symptoms of AN respectively.

A second family-based cohort was composed of 41 RAN trios of German origin, the probands being assessed with the Composite International Diagnostic Interview (CIDI) (Robins, *et al* 1988). The clinical features of the two family-based cohorts and subgroups are described in Table 1.

A third sample was composed of young women (mean age  $20.30 \pm 0.05$  years) with Caucasian origin recruited for a genetic analysis of addictive behaviours, including eating disorders (SAGE study) (Le Strat, *et al* 2009). Eating disorders were assessed using the SCOFF scale, a brief and reliable questionnaire developed as a screening test of eating

disorders in the general population with a sensitivity of 84.6% and a specificity of 89.6% (Hill, *et al* 2009). Participants were asked the following questions: 1. Do you make yourself sick because you feel uncomfortably full? 2. Do you worry you have lost control over how much you eat? 3. Have you recently lost more than 6 kg in a 3 month period? 4. Do you believe yourself to be fat when others say you are too thin? 5. Would you say that food dominates your life?

Participants were considered as having an eating disorder if they had a SCOFF score equal or above 2 (n=126), and were considered as control if they had a SCOFF score lower than 2 (n=567).

### ***SNPs and Genotyping***

Genomic DNA from probands and parents was extracted from peripheral blood leukocytes. Thirteen SNPs encompassing *ESR1* gene and four SNPs within *ESR2* gene were screened using TaqMan SNP genotyping assays (Applied Biosystems, Les Ullis, France). The selection of SNPs is based on tagged SNPs that depict the haplotype blocks across the genes, according to available databases (Perlegen and Hapmap) and positive associations with diseases.

Details of SNPs are indicated in Table 2 and Supplementary Table 2 and their position within genes are shown on Figure 1 and Supplementary Figure 1 for *ESR1* and *ESR2* genes, respectively. A total of 362 AN patients and 613 parents were genotyped. Furthermore, 30 samples were genotyped in duplicate to assess the accuracy of the allelic call. SNP rs3798577 was genotyped using SNPLEX technology Applied Biosystems, Les Ullis, France) for 693 women from the population-based sample (Tobler, *et al* 2005).

### ***Statistical analysis***

Comparisons of clinical features between cohorts and subsets were performed using Student's t-test and Chi-square test. Hardy-Weinberg equilibrium tests, linkage disequilibrium D' values and minor allele frequencies were computed using Haploview 4.1 software (Barrett, *et al* 2005). Transmission disequilibrium tests for SNPs and haplotypes were carried out with FBAT and PLINK programs (Horvath, *et al* 2001; Purcell, *et al* 2007). Parental origin of the association was calculated using ASPEX 2.5 package (Hinds and Risch, 1996). Gene-gene interaction was computed using the gene-based test of the PLINK program. The power of sample size for association tests was calculated using the Genetic Power Calculator program (<http://statgen.iop.kcl.ac.uk/gpc/>) (Purcell, *et al* 2003). To take account the linkage disequilibrium between each SNP, we computed correction for multiple testing for SNPs using Single Nucleotide Polymorphism Spectral Decomposition (SNPSpD) (<http://gump.qimr.edu.au/general/daleN/SNPSpD/>) (Nyholt, 2004).

## RESULTS

### *Association of ESR1 gene with AN and RAN subset*

We first genotyped 17 SNPs, encompassing *ESR1* and *ESR2* genes, in a French cohort of 321 AN families. All SNPs analyzed in this study were in Hardy-Weinberg equilibrium in the patients and parents. No discrepancy was observed in the genotyping of 30 duplicated samples. Two families were excluded due to Mendelian inheritance errors in the paternal transmissions.

Using transmission disequilibrium tests (TDTs), we found a significant over-transmission in AN with *ESR1* SNPs rs726281 ( $p=0.024$ , odds ratio [OR]=1.5, 95% confidence interval [95%CI]=1-2) and rs2295193 ( $p=0.024$ , OR=1.4, 95%CI=1-1.8), (Table 2). We showed that RAN and BPAN subjects differed significantly for several clinical variables, particularly regarding the age of onset of anorexia and the minimum lifetime BMI (Table 1). Therefore, we performed TDT analyses according to the RAN and BPAN subsets. Three *ESR1* SNPs were significantly over-transmitted in the RAN subset, rs726281 ( $p=0.005$ , OR=2.1, 95%CI=1.2-3.6), rs3798577 ( $p=0.021$ , OR=1.6, 95%CI=1.1-2.3) and rs2295193 ( $p=0.007$ , OR=1.7, 95%CI=1.2-2.5) (Table 3). In contrast, no SNP was associated with the BPAN subgroup (Supplementary Table 1).

No preferential transmission was found for the *ESR2* SNPs in any of the AN subgroups (Supplementary Table 2). Furthermore, only one haplotype block encompasses the 112 kb of the *ESR2* gene that renders unlikely the association between *ESR2* gene with AN, or with the RAN or BPAN subsets (Supplementary Figure 1).

No significant epistatic association was found for combinations of *ESR1* and *ESR2* SNPs and the AN cohort, neither for the RAN nor for the BPAN subgroups (data not shown).

### ***Haplotype association in the RAN subset***

Linkage disequilibrium analysis of pairwise SNPs revealed several haplotype blocks across the 296 kb of *ESR1* gene (Figure 1c). Over-transmissions in the RAN subset were found for common haplotypes based on five SNPs (Block 1: rs726281\*A-rs3020407\*A-rs17080994\*T-rs2982712\*T-rs3020371\*C, 0.503 frequency,  $p=0.06$ ), three SNPs (Block 2: rs2228480\*G-rs3798577\*T-rs2295193\*G, 0.410 frequency,  $p=0.002$ ), and the eight SNPs of the two blocks merged (rs726281\*A-rs3020407\*A-rs17080994\*T-rs2982712\*T-rs3020371\*C-rs2228480\*G-rs3798577\*T-rs2295193\*G, 0.230 frequency,  $p=6.10^{-6}$ ). This 8-SNPs haplotype presents an odds ratio of 3.1 (95%CI=1.8–5.1) for RAN.

### ***Parental origin of association***

In addition, we analyzed the parental origin of the association with RAN subset. Considering the combined TDT of the 13 *ESR1* SNPs, we detected a highly significant excess of paternal transmission ( $p=9.10^{-7}$ ) and a significant excess of maternal transmission ( $p=0.0004$ ). More precisely in RAN, a paternal over-transmission of four SNPs (rs726281  $p=0.024$ , rs2228480  $p=0.05$ , rs3798577  $p=0.008$  and rs2295193  $p=0.004$ ) and a maternal over-transmission of rs726281 only ( $p=0.041$ ) were observed (Table 3). Interestingly, the rs726281 over-transmitted by both parents is located in haplotype block 1, while the three SNPs solely transmitted in excess by the father constitute block 2 (Figure 1c). Regarding the 8-SNPs haplotype in RAN, in comparison to the maternal over-transmission ( $p=0.02$ , OR=2.1, CI95=1.1–3.8), we found a higher statistical significance for the paternal over-transmission ( $p=3.10^{-5}$ , OR=3.7, 95%CI=1.9–7.3) (Figure 1a).

***ESR1 association with an independent RAN German cohort***

As a second step, we attempted to replicate the association by genotyping *ESR1* SNPs in an independent German cohort of 41 RAN trios (Table 4). Although no allele was transmitted in excess in the probands of this replication cohort, we found a preferential paternal over-transmission of the same SNP which was initially associated in our sample, namely the rs726281 ( $p=0.025$ ), and a trend of significance for rs3798577 ( $p=0.059$ ), conferring a higher risk for RAN in this sample ( $OR=3.0$ ,  $95\%CI=1.1-8.3$ , and  $OR=2.6$ ,  $95\%CI=0.9-7.3$ , respectively).

***ESR1 association with eating disorders in an independent population-based sample***

In a population-based sample of 693 young French Caucasian women (mean age  $20.30 \pm 0.05$  years), tagged-SNP rs3798577 was found associated with the presence of eating disorders (Additive model:  $p=0.008$ ) (Table 5).

## DISCUSSION

In the current study, we found an association between *ESR1* gene and AN, mainly observed in the restrictive subtype of anorexia nervosa. We showed no association of *ESR2* with AN, nor with RAN or BPAN subgroups. Furthermore, we did not identified *ESR1-ESR2* gene-gene interaction with either AN or restrictive subtype.

Only two works of AN with *ESR1* and *ESR2* genes were previously published (Rosenkranz, *et al* 1998; Eastwood, *et al* 2002). The initial study has screened for mutation in *ESR2* gene (Rosenkranz, *et al* 1998) and found different distribution of the variant G1082A (rs1256049) in AN compared to BN or to obese/underweight subjects. In the second study, no association was reported between AN and 3 markers, or haplotypes, of *ESR1* gene, but a significant association was reported with the rs1256049 SNP of *ESR2* (Eastwood, *et al* 2002). Furthermore, an association was also reported with rs4986938 and rs928554 (but not with rs1256049) in bulimia nervosa and eating disorders not otherwise specified (Nilsson, *et al* 2004). In the present study, no association was found between *ESR2* SNPs, which are in linkage disequilibrium ( $D' > 0.9$ ) with rs1256049 according to databases, and AN, RAN or BPAN subsets. Noteworthy, our screening of 321 AN families using the transmission disequilibrium test was more powerful ( $1-\beta = 89\%$ ) than the two previous case-control studies ( $1-\beta = 18\%$  and  $63\%$ , respectively) and distinguished RAN and BAN.

We reported a strong association of specific SNPs and haplotypes of the *ESR1* gene and AN, and RAN subgroup, with a preferential paternal over-transmission. While we genotyped a large cohort of families and performed transmission disequilibrium tests that reduce the sex and ethnic stratification bias, the probability of a false positive finding in the present study cannot be excluded due to the number of tests performed. After a Bonferroni

correction taking into account the 13 SNPs within *ESR1* gene for the RAN subgroup, we found only trends of association for two SNPs, rs726281  $p_{\text{corrected}}=0.065$  and rs2295193  $p_{\text{corrected}}=0.091$ , but still observed a strong statistical significance for the 8-SNPs haplotype ( $p_{\text{corrected}}=0.00008$ ). It must be kept in mind that the Bonferroni correction is relevant for independent tests. Given that linkage disequilibrium between the 13 SNPs are mainly above zero in our sample, this correction is probably over-conservative. In order to assess a more appropriate p-value corrected for multiple testing for single SNP association, we carried out the Single Nucleotide Polymorphism Spectral Decomposition (SNPSpD) method (Nyholt, 2004). Significance threshold required is a  $p<0.0051$  (rs726281) and effective number of independent SNP is 10 instead of 13. Thus, we found a significant association for rs726281  $p_{\text{corrected}}=0.049$  and a trend for rs2295193  $p_{\text{corrected}}=0.07$ .

The three SNPs of *ESR1* associated with RAN in our sample (rs726281, rs3798577 and rs2295193) are located in introns and 3' UTR region. To date, they have no known functional consequences (Ascenzi, *et al* 2006). However, the associated haplotype block of 8 SNPs covers the C terminal E/F region of ESR1 protein, which corresponds to the ligand fixation domain of nuclear receptor. So, it is tempting to speculate that the association of these SNPs with RAN could suggest changes of ligand fixation in RAN patients.

We also attempted to replicate our results in an independent German cohort of 41 RAN trios, but failed to confirm significant associations between *ESR1* SNPs and RAN. Surprisingly, participants in the German RAN cohort showed a significant higher mean of minimal lifetime BMI compared to the French RAN cohort, while they were significantly younger and had shorter disease duration. As up to half of the restrictive AN patients cross-over to binge eating/purging subtype in a 7 years follow-up study (Eddy, *et al* 2008), this difference may explain the absence of replication in the German sample, since, in the latter,



young RAN patients could still switch to the BPAN phenotype. While this difference between the two cohorts decreases any chances of replication, we were able to observe the excess of paternal transmission of the main SNP associated with anorexia nervosa in the first sample. Due to a small sample size, the RAN German cohort provided a reduce power (power=33%) compared to the RAN French cohort (power=59%) that can explain the low significance of association.

Finally, we tested a tagged-SNP of the *ESR1* gene in a large population based sample, and found an association with eating disorders, as defined by a SCOFF score equal or above 2. However, it should be noted that the SCOFF scale is a screening tool for eating disorders rather than a diagnostic instrument for AN, and therefore no direct comparison should be made between the two first clinical samples and this population based sample. At most, this epidemiological sample gives indirect evidence for replication, which is usually regarded as needed for genetic association studies in complex disorders.

There are several limitations of this study. Firstly, the instruments assessing the eating disorders are different in the three cohorts. French and German trios families were assessed with semi-structured interview, respectively, DIGS and CIDI, while the self-evaluation SCOFF scale was used to screen epidemiological eating disorders in a large woman population sample. Secondly, the sample size of the German trios is small with a shorter disease evolution period, although a cohort with a larger sample size and a disease duration equivalent to the French cohort is recommended to replicate our results. Thirdly, the three SNPs of *ESR1* (rs726281, rs3798577 and rs2295193), associated with RAN, have no known functional consequences up to now and are located in non-coding regions. These SNPs required a functional analysis regarding their potential effects of associated alleles in *ESR1* receptor proprieties.

Although there is a lack of report on the genetic aspects of hormonal status as a risk factor for anorexia nervosa, recent investigations suggest that some hormonal environment might be associated with eating disorders (Procopio and Marriott, 2007; Young, 1991). Observations between same-sex and opposite-sex twins allowed to find a high AN prevalence in adult life in female compares to male, suggesting an influence of intrauterine exposure level to sex hormone (Procopio and Marriott, 2007). Interestingly, significant deficiency of serum levels for estradiol, the ligand of estrogen receptor, has been described in AN patients compared to controls, and a lower concentration in restrictive subtype rather than the binge eating/purging subtype has been reported (Ohwada, *et al* 2007). It is tempting to speculate that the association between *ESR1* gene and RAN may lead to specific biological effects, such as a modified expression and/or an altered function of the estrogen receptor alpha. Furthermore, the paternal transmission of the association that we observed also supports a genetic imprinting, which might be influenced by sex hormones. Also, it is important to consider DNA methylation in psychiatric disorder because it could help to the understanding of mechanism of the pathology and could constitute potential therapeutic targets. Acute administration of a selective ER alpha agonist leads to ovariectomized rats that showed a decrease in daily food intake and body weight (Santollo, *et al* 2007). Furthermore, in female mice, agonists of ER receptors produce different effects on social learning of food preferences (Clipperton, *et al* 2008). Then, ER alpha antagonist treatment failed to reduce food intake decrease in chemically ovariectomized rats (Santollo and Eckel, 2009). Finally, in female rat, the level of *ESR1* protein expression and estrogen sensitivity are modulated by oxytocin (Perry, *et al* 2009). Thus, it is difficult up to now to suggest a pharmacological treatment for AN. Further functional studies are needed to

decipher the biological effect of the association identified in our study which may influence the number or affinity of ESR1 receptor in restrictive AN patients.

Our study suggests evidences that common variants in *ESR1* gene are associated to eating disorders, and more specifically restrictive anorexia nervosa, highlighting the possible involvement of estrogenic hormonal pathway in anorexia nervosa that might open novel avenues of neuroendocrinopharmacological approaches in this neuropsychiatric disorder.

**DISCLOSURE/CONFLICTS OF INTEREST**

Authors declare no conflict of interest.

## **ACKNOWLEDGMENTS**

Overall, we acknowledge the kind assistance of the families, students and patients who participated in the studies. This work received grants from EC Framework V "Factors in Healthy Eating" (a consortium coordinated by Janet Treasure and David Collier), and from INRA/INSERM (4M406D). AV is supported by grants from "Région Ile-de-France". YLS is funded by the "Société Française de Tabacologie". The SAGE study was supported by grants from the Institut de Recherche sur les Boissons (IREB), and from the « Mission Interministérielle de Lutte contre la Drogue et la Toxicomanie » (MILDT).

## REFERENCES

- American Psychiatric Association (1994). *Diagnostic and statistical manual of mental disorders*, 4th ed., *Text Revision* (DSMIV-TR). Washington, DC: American Psychiatric Press.
- Baker JH, Maes HH, Lissner L, Aggen SH, Lichtenstein P, Kendler KS (2009). Genetic risk factors for disordered eating in adolescent males and females. *J Abnorm Psychol* **118**: 576-586.
- Bao AM, Hestiantoro A, Van Someren EJ, Swaab DF, Zhou JN (2005). Colocalization of corticotropin-releasing hormone and oestrogen receptor-alpha in the paraventricular nucleus of the hypothalamus in mood disorders. *Brain* **128**: 1301-1313.
- Barrett JC, Fry B, Maller J, Daly MJ (2005). Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* **21**: 263-265.
- Brambilla F, Monteleone P, Bortolotti F, Dalle Grave R, Todisco P, Faravo A, et al (2003). Persistent amenorrhoea in weight-recovered anorexics: psychological and biological aspects. *Psychiatry Res* **118**: 249-257.
- Clipperton AE, Spinato JM, Chernets C, Pfaff DW, Choleris E (2008). Differential effects of estrogen receptor alpha and beta specific agonists on social learning of food preferences in female mice. *Neuropsychopharmacology* **33**: 2362-2375.
- Couse JF, Korach KS (1999). Estrogen receptor null mice: what have we learned and where will they lead us? *Endocr Rev* **20**: 358-417.
- Culbert KM, Breedlove SM, Burt SA, Klump KL (2008). Prenatal hormone exposure and risk for eating disorders: a comparison of opposite-sex and same-sex twins. *Arch Gen Psychiatry* **65**: 329-336.

Dagnault A and Richard D (1997). Involvement of the medial preoptic area in the anorectic action of estrogens. *Am J Physiol* **272**: R311-317.

Eastwood H, Brown KM, Markovic D, Pieri LF (2002). Variation in the ESR1 and ESR2 genes and genetic susceptibility to anorexia nervosa. *Mol Psychiatry* **7**: 86-89.

Eddy KT, Keel PK, Dorer DJ, Delinsky SS, Franko DL, Herzog DB (2002). Longitudinal comparison of anorexia nervosa subtypes. *Int J Eat Disord* **31**: 191-201.

Eddy KT, Dorer DJ, Franko DL, Tahlilani K, Thompson-Brenner H, Herzog DB (2008). Diagnostic crossover in anorexia nervosa and bulimia nervosa: implications for DSM-V. *Am J Psychiatry* **165**: 245-250.

Foulon C, Guelfi JD, Kipman A, Adès J, Romo L, Houdeyer K, et al (2007). Switching to the bingeing/purging subtype of anorexia nervosa is frequently associated with suicidal attempts. *Eur Psychiatry* **22**: 513-519.

Garner DM, Olmsted MP, Bohr Y, Garfinkel PE (1982). The eating attitudes test: psychometric features and clinical correlates. *Psychol Med* **12**: 871-878.

Garner DM, Olmsted MP, Polivy J (1983). Development and validation of a multidimensional eating disorder inventory for anorexia nervosa and bulimia. *Int J Eat Disord* **2**: 15-34.

Gershon ES, Schreiber JL, Hamovit JR, Dibble ED, Kaye W, Nurnberger JI Jr, et al (1984). Clinical findings in patients with anorexia nervosa and affective illness in their relatives. *Am J Psychiatry* **141**: 1419-1422.

Gorwood P, Kipman A, Foulon C (2003). The human genetics of anorexia nervosa. *Eur J Pharmacol* **480**: 163-170.

- Grice DE, Halmi KA, Fichter MM, Strober M, Woodside DB, Treasure JT, et al (2002). Evidence for a susceptibility gene for anorexia nervosa on chromosome 1. *Am J Hum Genet* **70**: 787-792.
- Harris EC, Barraclough BM (1994). Suicide as an outcome for medical disorders. *Medicine (Baltimore)* **73**: 281-296.
- Hill LS, Reid F, Morgan JF, Lacey JH (2009). SCOFF, the development of an eating disorder screening questionnaire. *Int J Eat Disord* in press.
- Hinds DA and Risch N (1996). The ASPEX package: affected sib-pair exclusion mapping. <http://aspexsourcefor-genet/>.
- Hoek HW (2006). Incidence, prevalence and mortality of anorexia nervosa and other eating disorders. *Curr Opin Psychiatry* **19**: 389-394.
- Horvath S, Xu X, Laird NM (2001). The family based association test method: strategies for studying general genotype--phenotype associations. *Eur J Hum Genet* **9**: 301-306.
- Hudson JI, Hiripi E, Pope HG Jr, Kessler RC (2007). The prevalence and correlates of eating disorders in the National Comorbidity Survey Replication. *Biol Psychiatry* **61**: 348-358.
- Le Strat Y, Ramoz N, Horwood J, Falissard B, Hassler C, Romo L, Choquet M, Fergusson D, Gorwood P (2009). First positive reactions to cannabis constitute a priority risk factor for cannabis dependence. *Addiction* in press.
- Licinio J, Wong ML, Gold PW (1996). The hypothalamic-pituitary-adrenal axis in anorexia nervosa. *Psychiatry Res* **62**: 75-83.
- Milos G, Spindler A, Hepp U, Schnyder U (2004). Suicide attempts and suicidal ideation: links with psychiatric comorbidity in eating disorder subjects. *Gen Hosp Psychiatry* **26**: 129-135.



Morison IM, Ramsay JP, Spencer HG (2005). A census of mammalian imprinting. *Trends Genet* **21**: 457-465.

Musatov S, Chen W, Pfaff DW, Kaplitt MG, Ogawa S (2006). RNAi-mediated silencing of estrogen receptor {alpha} in the ventromedial nucleus of hypothalamus abolishes female sexual behaviors. *Proc Natl Acad Sci U S A* **103**: 10456-10460.

Musatov S, Chen W, Pfaff DW, Mobbs CV, Yang XJ, Clegg DJ, et al (2007). Silencing of estrogen receptor alpha in the ventromedial nucleus of hypothalamus leads to metabolic syndrome. *Proc Natl Acad Sci U S A* **104**: 2501-2506.

Nilsson M, Naessen S, Dahlman I, Linden-Hirschberg A, Gustafsson JA, Dahlman-Wright K (2004): Association of estrogen receptor beta gene polymorphisms with bulimic disease in women. *Mol Psychiatry* **9**: 28-34.

Nurnberger JI Jr, Blehar MC, Kaufmann CA, York-Cooler C, Simpson SG, Harkavy-Friedman J, et al (1994). Diagnostic interview for genetic studies. Rationale, unique features, and training. NIMH Genetics Initiative. *Arch Gen Psychiatry* **51**: 849-859; discussion 863-844.

Nyholt DR (2004). A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with Each Other. *Am J Hum Genet* **74**: 765-769.

Ohwada R, Hotta M, Sato K, Shibasaki T, Takano K (2007). The relationship between serum levels of estradiol and osteoprotegerin in patients with anorexia nervosa. *Endocr J* **54**: 953-959.

Osterlund MK, Gustafsson JA, Keller E, Hurd YL (2000). Estrogen receptor beta (ERbeta) messenger ribonucleic acid (mRNA) expression within the human forebrain: distinct distribution pattern to ERalpha mRNA. *J Clin Endocrinol Metab* **85**: 3840-3846.

- Osterlund MK and Hurd YL (2001). Estrogen receptors in the human forebrain and the relation to neuropsychiatric disorders. *Prog Neurobiol* **64**: 251-267.
- Perry AN, Paramadilok A, Cushing BS (2009). Neonatal oxytocin alters subsequent estrogen receptor alpha protein expression and estrogen sensitivity in the female rat. *Behav Brain Res* **205**: 154-161.
- Powell E, Xu W (2008). Intermolecular interactions identify ligand-selective activity of estrogen receptor alpha/beta dimmers. *Proc Natl Acad Sci USA* **105**: 19012-19017.
- Procopio M and Marriott P (2007). Intrauterine hormonal environment and risk of developing anorexia nervosa. *Arch Gen Psychiatry* **64**: 1402-1407.
- Purcell S, Cherny SS, Sham PC (2003). Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* **19**: 149-150.
- Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* **81**: 559-575.
- Raevuori A, Kaprio J, Hoek HW, Sihvola E, Rissanen K, Keski-Rahkonen A (2008). Anorexia and bulimia nervosa in same-sex and opposite-sex twins: lack of association with twin type in a nationwide study of Finnish twins. *Am J Psychiatry* **165**: 1604-1610.
- Ramoz N, Versini A, Gorwood P (2007). Eating disorders: an overview of treatment responses and the potential impact of vulnerability genes and endophenotypes. *Expert Opin Pharmacother* **8**: 2029-2044.
- Robins LN, Wing J, Wittchen HU, Helzer JE, Babor TF, Burke J, et al (1988). The Composite International Diagnostic Interview. An epidemiologic Instrument suitable for use in conjunction with different diagnostic systems and in different cultures. *Arch Gen Psychiatry* **45**: 1069-1077.

- Rosenkranz K, Hinney A, Ziegler A, Hermann H, Fichter M, Mayer H, et al (1998). Systematic mutation screening of the estrogen receptor beta gene in probands of different weight extremes: identification of several genetic variants. *J Clin Endocrinol Metab* **83**: 4524-4527.
- Santollo J, Wiley MD, Eckel LA (2007). Acute activation of ER alpha decreases food intake, meal size, and body weight in ovariectomized rats. *Am J Physiol Regul Integr Comp Physiol* **293**: R2194-2201.
- Santollo J, Eckel LA (2009). Effect of a putative ERalpha antagonist, MPP, on food intake in cycling and ovariectomized rats. *Physiol Behav* **97**: 193-198.
- Tobler AR, Short S, Andersen MR, Paner TM, Briggs JC, Lambert SM, et al (2005). The SNPLEX genotyping system: a flexible and scalable platform for SNP genotyping. *J Biomol Tech* **16**: 398-406.
- Van de Stolpe A, Slycke AJ, Reinders MO, Zomer AW, Goodenough S, Behl C, et al (2004). Estrogen receptor (ER)-mediated transcriptional regulation of the human corticotropin-releasing hormone-binding protein promoter: differential effects of ERalpha and ERbeta. *Mol Endocrinol* **18**: 2908-2923.
- Wade GN, Gray JM (1979). Gonadal effects on food intake and adiposity: a metabolic hypothesis. *Physiol Behav* **22**: 583-593.
- Wilson ME, Westberry JM, Prewitt AK (2008). Dynamic regulation of estrogen receptor-alpha gene expression in the brain: a role for promoter methylation? *Front Neuroendocrinol* **29**: 375-385.
- Young JK (1991). Estrogen and the etiology of anorexia nervosa. *Neurosci Biobehav Rev* **15**: 327-331.

Zehr JL, Culbert KM, Sisk CL, Klump KL (2007). An association of early puberty with disordered eating and anxiety in a population of undergraduate women and men. *Horm Behav* **52**: 427-435.

### Figure legends

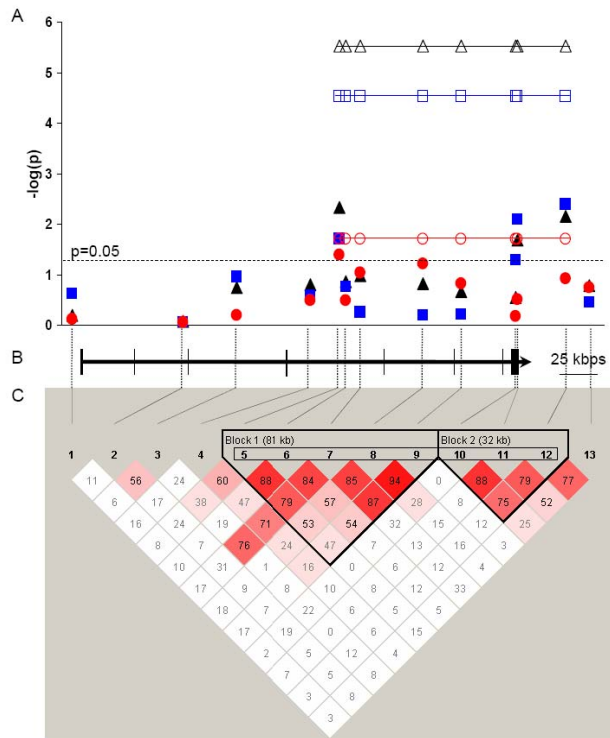
#### **Figure 1: ESR1 SNPs, mapping and association with restrictive anorexia nervosa**

(A) Association between restrictive anorexia nervosa and SNPs (filled symbol) or haplotypes (open symbol) for combined parental (black triangle), maternal (red circle) and paternal (blue square) transmissions. The strongest association is observed for the 8-SNPs haplotype ( $p = 6.10^{-6}$ ). (B) Genomic organization of *ESR1* gene (bars correspond to exons and arrowhead indicates the orientation of transcription) and position of the 13 encompassing SNPs (interrupted bars) at scale. (C) Pairwise linkage disequilibrium of SNPs. Haplotype blocks 1 and 2 are indicated. The haplotype of 8-SNPs is boxed.

#### **Supplementary Figure 1: Mapping of the ESR2 gene and SNPs and linkage disequilibrium**

(A) Genomic organization of *ESR2* gene (bars correspond to exons and arrowhead indicates the orientation of transcription) and position of the 4 encompassing SNPs (interrupted bars) at scale. The two alternative first exons are indicated by a and b. (B) Pairwise linkage disequilibrium of SNPs. Note that only one haplotype block encompasses *ESR2* gene.

**Figure 1**



## Tables

**Table 1: Demographic and clinical data for the French and German family-based cohorts and the French RAN and BPAN subgroups**

Population	French			German
Disorder	AN	RAN	BPAN	RAN
Families number (N)	321	153	154	41
Female (%)	97	97	97	95
Age (years)	22.3 ± 7.4	19.4 ± 6.5	25.2 ± 7.2 <sup>a</sup>	16.0 ± 1.9 <sup>b</sup>
Age of onset of anorexia (years)	16.2 ± 4.5	15.6 ± 4.4	16.8 ± 4.4 <sup>a</sup>	14.6 ± 1.2
Duration of anorexia (years)	6.1 ± 6.0	3.9 ± 4.8	8.3 ± 6.2 <sup>a</sup>	1.5 ± 1.3 <sup>b</sup>
BMI minimum (kg/m <sup>2</sup> )	13.6 ± 2.2	12.9 ± 1.7	14.4 ± 2.2 <sup>a</sup>	13.5 ± 0.2 <sup>b</sup>
Major depressive episode (%)	68	68	69	n.a.
Suicide attempt (%)	28	9	47 <sup>a</sup>	n.a.
Alcohol and drug abuse/dependence (%)	6	2	11 <sup>a</sup>	n.a.

EAT <sup>c</sup> Dieting	16.4 ± 10.6	13.4 ± 10.2	19.5 ± 10.3 <sup>a</sup>	n.a.
EAT Bulimia	7.6 ± 6.0	4.6 ± 4.3	10.6 ± 5.8 <sup>a</sup>	n.a.
EAT Oral control	7.4 ± 5.3	7.7 ± 5.2	7.2 ± 5.3	n.a.

---

Data are indicated as N, % or mean ± standard deviation. <sup>a</sup>Significant difference between RAN and BPAN from the French cohort. <sup>b</sup>Significant difference between French RAN subset and German RAN cohort. <sup>c</sup>Eating Attitude Test. Significant difference indicates a p value inferior at 0.05 in one-way analysis of variance or t-test. Not available, n.a.



**Table 2: Transmission disequilibrium test of *ESRI* SNPs with AN in the French family-based cohort**

SNP#	Markers	Position	MAF <sup>a</sup>	TDT			
				OT <sup>b</sup>	T:U <sup>c</sup>	<i>p</i>	OR [CI <sub>95</sub> ] <sup>d</sup>
1	rs488133	152167137	T (0.39)	C	102:102	1	1 [0.8-1.3]
2	rs11155819	152241052	C (0.31)	C	105:100	0.727	1.1 [0.8-1.4]
3	rs12199722	152276593	G (0.32)	G	99:88	0.421	1.1 [0.8-1.5]
4	rs1884051	152324972	G (0.3)	A	81:71	0.417	1.1 [0.8-1.6]
5	rs726281	152344271	G (0.26)	A	85:58	<b>0.024<sup>e</sup></b>	1.5 [1.0-2.0]
6	rs3020407	152348954	G (0.29)	A	95:76	0.146	1.3 [0.9-1.7]
7	rs17081994	152358440	C (0.09)	T	36:23	0.091	1.6 [0.9-2.6]
8	rs2982712	152399872	C (0.39)	T	106:95	0.438	1.1 [0.8-1.5]
9	rs3020371	152425513	T (0.31)	C	93:87	0.655	1.1 [0.8-1.4]
10	rs2228480	152461788	A (0.17)	G	55:50	0.626	1.1 [0.8-1.6]
11	rs3798577	152462823	C (0.46)	T	116:91	0.082	1.3 [1.0-1.7]
12	rs2295193	152494787	G (0.47)	G	117:85	<b>0.024</b>	1.4 [1.0-1.8]

13	rs2252837	152510513	T (0.35)	C	106:87	0.171	1.2 [0.9-1.6]
----	-----------	-----------	----------	---	--------	-------	---------------

<sup>a</sup>MAF: Minor allele and its frequency in the parentheses. <sup>b</sup>OT: Over-transmitted allele. <sup>c</sup>T:U: Transmitted versus untransmitted allele. <sup>d</sup>OR [CI<sub>95</sub>]: odds ratio and the 95% confidence interval. <sup>e</sup>Significant *p* values are indicated in bold.

**Table 3: Transmission disequilibrium test of *ESRI* SNPs with restrictive AN subset of French family-based cohort**

SNP#	Markers	TDT				Maternal TDT		Paternal TDT	
		OT <sup>a</sup>	T:U <sup>b</sup>	<i>p</i>	OR [CI <sub>95</sub> ] <sup>c</sup>	<i>p</i>	OR [CI <sub>95</sub> ]	<i>p</i>	OR [CI <sub>95</sub> ]
1	rs488133	C	56:51	0.629	1.1 [0.8-1.6]	0.758	.9 [0.5-1.7]	0.237	1.5 [0.8-2.9]
2	rs11155819	C	51:49	0.841	1 [0.7-1.5]	0.866	1.1 [0.5-2.1]	0.873	1.1 [0.6-2.0]
3	rs12199722	G	53:40	0.178	1.3 [0.9-2.0]	0.631	1.2 [0.6-2.2]	0.114	1.7 [0.9-3.2]
4	rs1884051	A	42:30	0.157	2.0 [1-3.8]	0.330	1.4 [0.7-2.6]	0.257	1.5 [0.7-3.3]
5	rs726281	A	48:24	<b>0.005<sup>d</sup></b>	2.1 [1.2-3.6]	<b>0.041</b>	2.2 [1.0-4.9]	<b>0.024</b>	2.3 [1.1-4.8]
6	rs3020407	A	52:38	0.140	1.4 [0.9-2.1]	0.317	1.4 [0.7-2.7]	0.170	1.6 [0.8-3.2]
7	rs17081994	T	20:11	0.106	1.8 [0.9-3.8]	0.090	2.4 [0.8-6.8]	0.564	1.4 [0.4-4.4]
8	rs2982712	T	61:46	0.147	1.3 [0.9-1.9]	0.064	1.8 [1.0-3.4]	0.647	1.2 [0.6-2.1]
9	rs3020371	C	53:41	0.216	1.3 [0.9-1.9]	0.150	1.6 [0.8-3.0]	0.622	1.2 [0.6-2.2]
10	rs2228480	G	31:23	0.276	1.3 [0.8-2.3]	0.670	.8 [0.4-1.9]	<b>0.050</b>	2.3 [1.0-5.2]
11	rs3798577	T	66:42	<b>0.021</b>	1.6 [1.1-2.3]	0.307	1.4 [0.8-2.4]	<b>0.008</b>	2.4 [1.2-4.7]
12	rs2295193	G	68:40	<b>0.007</b>	1.7 [1.2-2.5]	0.123	1.6 [0.9-3.0]	<b>0.004</b>	2.6 [1.3-5.3]

13	rs2252837	C	57:43	0.162	1.3 [0.9-2.0]	0.182	1.6 [0.8-3.1]	0.355	1.3 [0.7-2.5]
----	-----------	---	-------	-------	---------------	-------	---------------	-------	---------------

<sup>a</sup>OT: Over-transmitted allele. <sup>b</sup>T:U: Transmitted versus untransmitted allele. <sup>c</sup>OR [CI<sub>95</sub>]: odds ratio and the 95% confidence interval. <sup>d</sup>Significant *p* values are indicated in bold.

**Table 4: Transmission disequilibrium test of *ESRI* SNPs with restrictive AN of German family-based cohort**

SNP#	Markers	MAF <sup>a</sup>	Parental TDT				Maternal TDT			Paternal TDT		
			OT <sup>b</sup>	T:U <sup>c</sup>	<i>p</i>	OR [CI95] <sup>d</sup>	T:U	<i>p</i>	OR [CI95]	T:U	<i>p</i>	OR [CI95]
4	rs1884051	G (0.30)	G	20:15	0.398	1.3 [0.7-2.6]	09:09	1	1 [0.4-2.5]	11:06	0.225	1.8 [0.7-5.0]
5	rs726281	G (0.27)	G	23:14	0.139	1.6 [0.8-3.2]	08:09	0.808	0.9 [0.3-2.3]	15:05	<b>0.025<sup>e</sup></b>	3 [1.1-8.3]
6	rs3020407	G (0.33)	A	17:16	0.862	1.1 [0.5-2.1]	11:06	0.225	1.8 [0.7-5.0]	06:10	0.317	0.6 [0.2-1.7]
7	rs17081994	C (0.09)	C	08:05	0.405	1.6 [0.5-4.9]	01:02	0.564	0.5 [0-5.5]	06:02	0.157	3 [0.6-14.9]
9	rs3020371	T (0.32)	T	22:16	0.330	1.4 [0.7-2.6]	09:08	0.808	1.1 [0.4-2.9]	12:07	0.251	1.7 [0.7-4.4]
10	rs2228480	A (0.19)	G	15:13	0.705	1.2 [0.5-2.4]	08:06	0.593	1.3 [0.5-3.8]	08:07	0.796	1.1 [0.4-3.2]
11	rs3798577	C (0.40)	T	23:13	<i>0.096<sup>f</sup></i>	1.8 [0.9-3.5]	10:08	0.637	1.3 [0.5-3.2]	13:05	<i>0.059</i>	2.6 [0.9-7.3]
12	rs2295193	A (0.45)	-	21:21	1	1 [0.5-1.8]	11:11	1	1 [0.4-2.3]	10:10	1	1 [0.4-2.4]

<sup>a</sup>MAF: Minor allele and its frequency in the parentheses. <sup>b</sup>OT: Over-transmitted allele. <sup>c</sup>T:U: Transmitted versus untransmitted allele. <sup>d</sup>OR [CI<sub>95</sub>]:

odds ratio and the 95% confidence interval. <sup>e</sup>Significant *p* values are indicated in bold. <sup>f</sup>Trend of significance for *p* values are indicated in italic.

**Table 5: Association of *ESR1* rs3798577 tag-SNP with AN in a population-based sample of 693 French Caucasian women.**

	Genotype							
	CC		CT		TT		Total	
	N	%	N	%	N	%	N	%
SCOFF<2	128	22.6	284	50.1	155	27.3	567	100
SCOFF≥2	19	15.1	59	46.8	48	38.1	126	100
<i>p</i> value (Additive model) = <b>0.008</b>								

