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Biological, psychological and familial specific correlates in eating disorders at onset: a control-case study protocol (ANOBAS)

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ABSTRACT

Background. The complexity in the development of an eating disorder (ED) pose methodological challenges when addressing risk factors of this pathology. Pike et al. (2008) proposed to use a case-control design for this type of research. The eating disorders' risk factor study (ANOBAS) is a case-control study with three control groups aiming to evaluate several variables related to the onset of ED, thus the aim was to illustrate a new methodology proposal and to assess whether the chosen control groups are appropriate to research correlates on ED.

Methods. We used a case-control design of 50 female adolescents with ED at onset matched by age and their parents' socioeconomic status with 40 patients with an affective disorder, 40 patients with asthma pathology and 50 without pathology. Diagnoses were completed with K-SADS interview and an evaluation of biological, psychological, environmental and family correlates.

Results. Higher similarities were found between the ED group and the affective disorder group across psychological variables, whereas the similarities between the ED group and the asthma group were found at the familial level, as we expected. The biggest differences were found with the non-pathology group.

Conclusions. This rigorous research design allows investigating correlates associated specifically to the onset of an ED and the chosen control groups are suitable to investigate it.

Key words. Eating disorders, case-control study, correlates, onset, control groups.

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CORRELATOS BIOLÓGICOS, PSICOLÓGICOS Y FAMILIARES ESPECÍFICOS EN EL INICIO DE LOS TRASTORNOS ALIMENTARIOS: ESTUDIO CASO-CONTROL (ANOBAS)

RESUMEN

Introducción. La complejidad asociada al desarrollo de los trastornos alimentarios (TCA) supone un reto metodológico en la investigación sobre los factores de riesgo implicados en el trastorno, siendo el diseño caso-control el adecuado para investigarlos. El Estudio de Factores de Riesgo de los TCA (ANOBAS) es un estudio caso-control con tres grupos de control que tiene el objetivo de evaluar distintos correlatos relacionados con el inicio de un TCA. El objetivo de este estudio es mostrar una nueva propuesta metodológica de evaluación y comprobar si los grupos de control son adecuados para estudiar cuáles son los correlatos específicos.

Metodología. Se usó un estudio caso-control con 50 adolescentes con TCA al inicio de la patología, emparejadas por edad y estatus socioeconómico de los padres con 40 adolescentes con trastorno afectivo al inicio, 40 adolescentes con patología asmática y 50 adolescentes sin patología. El diagnóstico se realizó a través de la entrevista K-SADS y se hizo una evaluación de los correlatos biológicos, psicológicos y familiares.

Resultados. Como se esperaba, altas similitudes fueron encontradas entre el grupo TCA y el grupo con trastorno afectivo en los correlatos psicológicos, mientras que las similitudes entre el grupo TCA y el grupo con patología asmática fueron encontradas en el plano familiar. Las principales diferencias fueron encontradas con el grupo sin patología.

Conclusiones. Esta rigurosa propuesta metodológica permite investigar qué correlatos se asocian específicamente al inicio de un TCA, siendo los grupos de control seleccionados adecuados para investigarlo.

Palabras clave. trastornos alimentarios; estudio caso-control; correlatos; inicio; grupo de control.

INTRODUCCIÓN

Eating disorders (EDs) are severe psychiatric disorders associated with complications in medical and psychological conditions[1]. The study of risk factors associated with EDs carries inherent difficulties. Mainly, the general low prevalence rates as well as the complexity in the aetiology of the disorder significantly complicate the attainment of sound conclusions[2]. Other problems are the lack of differences between specific and general risk factors[3], the establishment of the period in which they occur[4] and the study of the interaction between variables following a biopsychosocial perspective[5].

Based on rigorous longitudinal and meta-analytic studies, some psychological risk factors have been identified in the development of an ED. Perfectionism and negative affectivity have been found to be important risk factors in different studies[6,7]. Although some studies have proposed that they are risk factors for ED in general, Tyrka et al.[8] have specified that negative affect predicts bulimia nervosa (BN) whereas perfectionism is a predictor of anorexia nervosa (AN). Another proposed risk factor is body dissatisfaction, which is distinguished as one of the most robust risk factors for the onset of AN[9]. Moreover, thin-ideal internalization has been found to be an ED risk predictor[10]. Jacobi and Fittig[11] found other risk factors, such as shape and weight concerns, dietary restraints, previous psychiatric disorders, obstetric complications and negative life events. In addition, Krug, Taborelli, Sallis, Treasure and Micali[12] noted associations between neonatal and obstetric complications, such as prematurity and instrumental delivery, and later ED symptomatology.

Regarding family variables, weight teasing and critical comments about eating from parents[13], as well as negative perception of parents' attitudes[14] and having a higher maternal education level[15] were some of the predictors that have been identified in longitudinal studies. However, in the McNight Longitudinal Risk Factor Study[16] and in the thirty-year cohort study of AN[17], none of these parental influences could be found.

Some biological variables have been related to the onset of ED, although it is difficult to know whether the biomarkers are prior to or a consequence of the course of development of an ED[18]. In this sense, patients tend to experience several hormone imbalances related to cortisol and sex hormones[19]. In addition, Monteleone and Maj (20) have found that some peptides, such as leptin, decrease in severe phases of AN.

Another way to investigate risk factors is through a case-control design, aiming to find proximal risk factors in a sufficient number of cases. This tends to be difficult in the follow up of longitudinal studies. Similar risk factors were found in case-control studies[2,21–25]. For example, perfectionism was found to be a risk factor for ED in general[22], only for AN[21] or only for BN[25]. Regarding negative affectivity, Machado et al.[2] did not find this relation with AN. Other family factors have been recorded in case-control studies. For instance, a family history of ED appears as the strongest predictor[24]. Family discord[21], high parental demands[23], negative attitudes regarding parents' shape and weight[2], history of abuse and parental depression[22] are other familial risk factors that have been reported. Even so, when the comparison included a psychiatric control group, some of these factors became unspecific[2].

Addressing these potential shortcomings, the aim of the ANOBAS study was to identify specific ED correlates based on an integrative perspective. Following the Kraemer et al.[4]'s risk factors classification, correlates are the kind of factors that can not demonstrate precedence over the outcome. To evaluate the specificity of these correlates, three specific control samples were used: an affective disorder group, an asthma pathology group and a non-pathology control group (not presenting the aforementioned pathologies).

The decision to include an affective disorder control group was based on the fact that both EDs and affective disorders share depression disorders as a common and frequent comorbidity[26]. Indeed, not only is depression associated with the later development of an ED[27], but it might also predict a future ED pathology[13]. Other authors, such as Ferreiro, Seoane, and Senra[28], suggest that both disorders may share epidemiological similarities and psychological risk factors. Regarding the inclusion of an asthma group, asthma has been classified as a psychosomatic disease, similarly to eating disorders[29]. Likewise, similarly to EDs, severe or moderate asthma has been associated with psychiatric, social and physical problems[30], although similarities between risk factors for the two pathologies are best observed amongst the patients' families, as they both require high levels of caregiving and thus, represent a significant impact on the physical and psychological wellbeing of the families[31]. In addition, there is an influence between the psychological adjustment of the parents and the child's response to asthma[32], as well as occur in ED[33]. Lastly, a non-pathology control group devoid of diagnosed pathologies allows for comparisons between EDs and a group that shares similar changes in the individual's biology, psychology and social environment[5]. Moreover, although around 30-70% of adolescents present some ED risk factors, less than 10% develop an ED[34].

Although similar studies have been published in recent years[2,21,25], the majority of these studies assess only the patient, instead of considering other informants

also. Lastly, no case-control study has included more than two control groups or adolescents at the onset of the ED pathology, nor have they used a bio-psycho-familial approach. The aim of the current study is to present a new methodology proposal based on an integrative approach and to assess whether the three chosen control groups are suitable to research specific correlates associated to the onset of an ED. Thus, we expected more similarities between the ED and affective disorders groups in the psychological variables, and more similarities between the ED and asthma groups in familial variables. We also predicted that most of the differences would be found with the non-pathology control group.

METHODS

Design

The design proposed in the study was a cross-sectional matched case-control study, with the aim of establishing differences between specific correlates of an ED group and three control groups. Four samples were matched by sex, age and socioeconomic status of the parents, following the Hollinshead Redlich Scale (35). Matching by age minimizes age-related bias and matching by socioeconomic status reduces the impact of this important cofounding variable[2]. The study design targeted firstly the year of the onset of the ED pathology so as to minimize differences of risk factor exposure time[22].

Participants and procedures

The participants were 180 teenager girls aged between 12 and 17, and their families, matched by sex and socioeconomic status. The sample was composed of 50 teenagers diagnosed with an eating disorder (ED group) and three control groups: 40 girls with affective disorders (AD group), 40 girls with an asthma pathology (AP group) and 50 girls without any of the previously mentioned disorders (NP group). Based on G-power analysis (36), a sample size of 40 or 50 is enough to reach good effect sizes. ED participants presented the following diagnoses: anorexia nervosa (AN) restrictive subtype (n=35; 70%); AN purgative subtype (n = 8; 16%) and other specified feeding and eating disorder (n=7; 14%).

For all groups, exclusion criteria were the presence of metabolic disorders that could affect the body mass index (BMI) and psychosis. In addition, exclusion criteria for the three control groups were having a BMI above 30 and below 18 or have an eating disorder. Inclusion criterion for the ED and AD group was presenting an early stage of the illness at first diagnosis (a year or less of illness duration). For the AP group, inclusion criteria included an asthma diagnosis, which should be first diagnosed before the age of 7 years and included at least three visits to an emergency service. AD and AP group must not have an ED diagnosis. For the NP group, the lack of any ED, AD or asthma pathology was considered as an inclusion criterion. Overall, nine participants were excluded after the assessment because of co-occurrence of ED or AD (n=2), co-occurrence of ED or AP (n=2), presence of psychosis (n=1), presence of a metabolic disorder (n=1) and ED pathology in NP group (n=3). Three NP matched participants dropped out the research (personal reasons).

The samples were recruited between 2012 and 2016. Short telephone interviews were conducted to confirm the sociodemographic variables and once informed consent was obtained, the cases were matched. This study was approved by the Autonomous University of Madrid Ethic Committee (Ref Code. R-0009/10) and by the Niño Jesus Hospital Ethic Research Committee (CEI 25-673).

ASSESSMENT

Diagnostic assessment

Current and lifetime psychiatric disorders were evaluated with the Kiddie-Schedule for Affective Disorders and Schizophrenia Interview (K-SADS-PL)[37]. The K-SADS-PL is a semi-structured interview developed to diagnose children and adolescents using DSM-IV Axis I diagnoses. Diagnoses were adapted to DSM-5[1]. To assess eating disorder pathologies, an Eating Disorders interview designed at the Hospital was used to evaluate eating disorders.

Clinical assessment: psychological, familial and biological variables.

A structured interview was carried out to assess socio-demographic variables and to complete the clinical history of each person. Questions about the pregnancy and birth, development, medical and psychiatric antecedents and any other treatments were asked.

The evaluation protocol is presented in Appendix A. It is a complex protocol with a battery of nine instruments for the adolescents and nine questionnaires for the parents, all the instruments had adequate psychometric validity across Spanish populations, as detailed in the Appendix. Regarding the biological assessment, a physical examination and laboratory analysis of blood markers related to nutritional and immunological status were assessed. An anthropometric examination (weight, height and body mass index (BMI) were completed with an evaluation of the participant's menstrual state.

Data analysis

Data were pseudonymised. Continuous variables were described using centralization indices. Categorical variables were described through percentages. The normality of the variables was verified through the Kolmogorov-Smirnov goodness-of-fit test. Univariate analyses were conducted to obtain differences between groups with their correspondent post-hoc comparison. As most of the variables did not fit to the normality assumption, the Friedman Test was used to compare all the groups at the same time, and later, the Wilcoxon Test was used to identify ED correlates. BMI standard deviation scores (BMI z-scores) were computed by comparing the children's BMI with the ideal BMI of the general population of the same sex and age[38].

The contrast between ED and the other control groups was conducted to identify the specific ED correlates. For this, different comparisons were carried out and Holm's Sequential Bonferroni Procedure[39], which deals with familywise error rates for multiple hypothesis tests, was applied. Moreover, standardized mean differences (effect sizes) were estimated in order to analyze whether the three control groups (AD, AP and NP) are suitable to research specific ED correlates.

Statistical analyses were carried out using the statistical software SPSS 21.0, version for Windows. Statistical analyses were performed with a significance level of 0.05.

RESULTS

Demographic characteristics

Demographic characteristics of the sample are described in Table 1. Results are presented for each group: ED, AD, AP, and NP. Similar results were found between groups for adolescents' age, socioeconomic status, as we expected, while similar results for marital status and psychiatric background of the father were found ($p > .05$), strong differences appeared for parents age and BMI z-scores ($p < .001$). In terms of BMI z-scores, the ED group obtained the lowest results, as expected due to the eating pathology. In addition, the most frequent marital status of the families was married, although this was less common in the AD group. Regarding psychiatric background, a maternal history of mental disorder was the most important difference between ED and AD group ($p = .004$). Similar results were found in relation with the grandparent's history of mental disorder ($p = .04$). Lastly, regarding academic performance, the ED group presented the highest achievement when compared with the three control groups ($p < .001$).

Clinical baseline results

Some results of the case-control study are presented in Table 2, divided into psychological variables of the adolescents and of the families. Biological variables, the presence of current comorbidity and the history of a mental disorder are presented in Table 3.

Regarding the psychological variables, the psychiatric groups (ED and AD) obtained overall higher results, especially regarding body dissatisfaction and obsessive symptoms with a slightly higher score in the AD group. In contrast, the ED group obtained higher scores in self-perfectionism. However, no differences between those groups were found on these variables. The only differences between ED and AD group were found in eating psychopathology ($p < .001$), higher in ED, and socially prescribed perfectionism ($p < .001$), higher in AD. In comparison to the non-psychiatric groups, differences were found in all the variables except in obsessive symptoms and socially prescribed perfectionism. Standardized mean differences (effect sizes) were considerably smaller for ED vs. AD comparison in front of the other groups (ED vs AP; ED vs NP) for all the psychological variables (except for socially prescribed perfectionism).

In terms of family variables, AD parents presented higher clinical problems, especially regarding obsessive symptoms of the mother ($p = .01$). However, ED parents had higher levels of depression of the mother when they were compared to the NP group ($p < .001$). Family cohesion reported by the fathers was lower than the mothers for all of the groups, whereas fewer differences were found on the reports about the adaptability of the family in both fathers and mothers. In addition, higher levels of cohesion were found in the ED group than in the AD group in fathers ($p < .001$) but no in mothers, whereas no differences were found between the groups in the type of families reported by fathers and mothers. Moderately balanced and balanced types of families were the most common styles for all the groups. Surprisingly, regarding the comparison with the AP group, no differences were found in any of the familial variables. Standardized mean differences (effect sizes) between those groups ranged from 0.09 (negligible effect) to 0.33 (small effect).

In terms of biological data, levels of cortisol, leptin and C3 appeared as good differential biomarkers in ED (see Table 3). In this manner, the mean for the ED group for levels of cortisol was significantly higher than the means for these variables in the rest of the groups ($p < .001$). Furthermore, the mean level of C3 in the ED group was significantly lower than the results in the other groups ($p < .002$). The level of leptin was also significantly lower in the ED group than in the AD and NP groups ($p < .001$). On the other hand, level of

Table 1 Differences in demographic characteristics between groups

	ED N=50	AD N=40	AP N=40	NP N=50			ED vs AD	ED vs AP	ED vs NP
					Friedman Test		Wilcoxon Test		
Adolescents	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**χ^2**	**p**	**p**	**p**	**p**
Age (12-17)	14.64 (1.41)	15.1 (1.55)	14.72 (1.73)	14.66 (1.32)	2.76	p=.43	-	-	-
Body Mass Index z-scores (BMI)	-1.85 (.75)	.48	.13 (.96)	-.40 (2.41)	81.41	**p<.001**	**p<.001**	**p<.001**	**p<.001**
	N (%)	**N (%)**	**N (%)**	**N (%)**					
Academic performance									
Very good	33 (66)	6 (15)	10 (25)	14 (28)					
Good	12(24)	9 (22.5)	23 (57.5)	20 (40)					
Regular	3 (6)	13 (32.5)	5 (12.5)	6 (12)	p<.001	**p<.001**	**p<.001**	**p=002**	**p=022**
Wrong	2 (4)	6 (15)	1 (2.5)	1 (2)					
Very wrong	0 (0)	6 (15)	1 (2.5)	1 (2)					
Parents	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**χ^2**	**p**	**p**	**p**	**p**
Father's age	47.63 (4.85)	48.32 (4.89)	46.91 (4.61)	51.02 (4.41)	17.86	**p<.001**	p=.56	**p<001**	**p<001**
Mother's age	45.47 (3.5)	45.73 (4.83)	45.64 (4.16)	48.3 (3.54)	74.39	**p<.001**	p=.88	**p<001**	**p<001**
	N (%)	**N (%)**	**N (%)**	**N (%)**					
Socioeconomic Status									
I	6 (12)	4 (10)	2 (4.9)	4 (8)					
II	4 (8)	7 (17.5)	6 (14.6)	3 (6)	17.13	p=.15	-	-	-
III	6 (12)	10 (25)	7 (17.1)	7 (14)					
IV	10 (20)	11 (27.5)	12 (29.3)	11 (22)					
V	24 (48)	8 (20)	11 (26.8)	25 (50)					
Marital status									
Married	39 (78)	24 (60)	34 (82.9)	39 (78)					
Divorce	8 (16)	6 (15)	4 (9.8)	6 (12)					
Separated	2 (4)	6 (15)	1 (2.4)	3 (6)		p=.26			
Single	0 (0)	3 (7.5)	0 (0)	0 (0)	18.06		-	-	-
Widowed	1 (2)	1 (2.5)	1 (2.4)	1 (2)					
Psychiatric background father- Yes	7 (14)	8 (20)	4 (9.8)	5 (10)	2.83	p=.42	-	-	-
Psychiatric background mother- Yes	20 (40)	20 (50)	12 (29.3)	13 (26)	18.24	**p=.03**	**p=.004**	p=.27	p=.07
Psychiatric background grandparents-Yes	16 (34)	17 (42.5)	6 (14.6)	17 (34)	10.5	**p=.02**	**p=.04**	p=.28	p=.58

Note. ED = Eating Disorders. AD = Affective Disorder. AP =Asthma Pathology. NP = Non-pathology group. M = Mean. SD = Standard Deviation. The significant values after Bonferroni correction are in bold.

C4 was similar between ED and the control groups (p= .11), but higher in the AD group.

Finally, current comorbidity was more frequent in ED, with 36% of the sample having a co-occurring disorder. This difference was only significant when the comparison included the non-psychiatric groups (p<.01). Nevertheless, a history of mental disorder in the past was more frequent in the AD group (45%), significantly higher than in the ED group (p<.001). Similar results were found between the non-psychiatric groups in current comorbidity and psychiatric backgrounds.

Table 2 Differences in psychological and familial variables between case-control groups: ANOBAS study

	ED [1] n=50	AD [2] n=40	AP [3] n=40	NP [4] n=50			ED vs AD	ED vs AP	ED vs NP	ED vs AD	ED vs AP	ED vs NP
					Friedman Test		Wilcoxon Test			Cohen`s d		
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	χ^2	p-valor	p-valor	p-valor	p-valor	d	d	d
Psychological variables in adolescents												
Body dissatisfaction (BSQ)	95.54 (45.02)	101.8 (47.46)	59.07 (24.79)	62.88 (27.12)	30.31	**p<.001**	p=.69	**p<.001**	**p<.001**	0.14	0.99	0.89
Eating psychopathology (EAT-26)	25.95 (18.06)	12.33 (10.51)	5.52 (3.94)	5.36 (6.85)	73.96	**p<.001**	**p<.001**	**p<.001**	**p<.001**	0.91	1.5	1.52
Obsessive symptoms (LOI)	9.10 (4.67)	10.00 (3.30)	6.82 (3.62)	7.40 (3.80)	15.31	**p=.007**	p=.16	p=.05[a]	p=.07	0.22	0.54	0.4
Perfectionism Self-directed (CAPS)	39.11 (10.95)	36.74 (8.31)	34.33 (8.71)	32.96 (7.81)	11.25	**p=.01**	p=.21	**p=.02**	**p=.01**	0.24	0.48	0.65
Perfectionism Socially prescribed (CAPS)	21.60 (8.97)	28.82 (38.38)	23.62 (7.82)	24.29 (7.83)	16.71	**p=.01**	**p<.001**	p=.21	p= .11	0.28	0.24	0.32
Family Variables												
Obsessive symptoms (OCI)												
Father	11.38 (8.23)	15.19 (12.03)	12.20 (9.65)	11.32 (6.77)	.156	p=.98	-	-	-	0.38	0.09	0.01
Mother	11.04 (10.86)	15.94 (9.19)	12.77 (8.38)	11.29 (7.78)	13.42	**p=.003**	**p=.01**	p=.09	p=.09	0.49	0.18	0.03
Depresión (BDI)												
Father	5.47 (4.25)	10.89 (11.04)	4.68 (4.13)	3.55 (2.83)	4.65	p=.19	-	-	-	0.68	0.19	0.54
Mother	7.94 (3.67)	11.32 (7.34)	7.62 (7.30)	5.28 (4.34)	23.74	**p=.01**	p=.03[a]	p=.48	**p<.001**	0.61	0.11	0.67
Funcionamiento familiar (FACES)												
Father cohesion	66.75 (9.7)	58.67 (7.70)	68.04 (6.66)	64.33 (7.48)	5.43	**p=.01**	**p<.001**	p=.21	p=.21	0.92	0.15	0.28
Father adaptability	52.95 (5.78)	50.21 (6.54)	52.04 (6.53)	51.26 (6.26)	1.57	p=.66	-	-	-	0.45	0.15	0.28
Father type	N %	N %	N %	N %								
Extreme	1 1.9	1 3.7	0 0	0 0								
Mid-range	4 7.7	5 18.5	1 4.5	6 12	χ^2	p						
Mod. balanced	18 34.6	17 63	12 54.5	21 42	13.05	p=.16	-	-	-	-	-	-
Balanced	22 42.3	4 14.8	9 40.9	12 78								
Mother cohesion	67.33 (9.57)	63.19 (8.87)	70.11 (6.91)	66.75 (6.91)	2.72	p=.44	-	-	-	0.45	0.33	0.07
Mother adaptability	52.83 (6.21)	51.25 (7.94)	52.24 (6.06)	52.28 (4.83)	.17	p=.83	-	-	-	0.23	0.1	0.1
Mother type	N %	N %	N %	N %								
Extreme	1 1.9	3 8.3	0 0	0 0								
Mid-range	7 13.5	8 13.9	1 4.8	3 6	χ^2	p						
Mod. balanced	18 34.6	16 44.4	6 28.6	26 52	14.97	p=.09	-	-	-	-	-	-
Balanced	23 44.2	12 33.3	14 66.7	19 38								

Note. ED = Eating Disorders. AD = Affective Disorder. AP =Asthma Pathology. NP = Non-pathology group. M = Mean. SD = Standard Deviation. The significant values after Bonferroni correction are in bold. a Although some statistical tests were statistically significant (p<.05), Holm-Bonferroni correction for multiple comparisons (Holm, 1979) showed that they could be significant by azar.

DISCUSSION

The aim of the present study was to show an innovative methodology proposal, which integrates a bio-psycho-familial assessment of correlates of eating disorders at onset along with a standardized comparison with control groups. Following recommendations on the study of the correlates in the ED by Jacobi et al.[3], the study was designed to target the earliest stages of the pathology, and therefore, the recruited ED participants were at what can be considered the onset of the illness.

Reviewing previous methodology designs used in ED correlates research, Fairburn et al.[23] perhaps pioneered one of the first case-control studies for ED. In this study, the authors recommended evaluating different correlates as well as taking into account the precedence and the interaction between these correlates to add robustness to the overall study design. There have been other studies[2,21,25] of note that have followed these recommendations. Both studies used a general psychiatric control group and non-psychiatric control group, but the mean illness duration with the ED was 10 years and only the patients were assessed. Both studies found similar results identifying specific correlates for ED. Thus, continuing this line of research, our study has sought to replicate and add to the previous body of ED correlates research.

Fine-tuning the recommended methodology, the current research seeks to prove that the chosen control groups are suitable control groups for the study of correlates on ED. As we hypothesized, the results found confirmed the strong similarities in psychological variables between ED and AD groups, as expected, regarding the high comorbidity between those disorders[26]. Body dissatisfaction as well as obsessive symptoms and self-directed perfectionism were similar between both pathologies. Whereas body dissatisfaction was found as an important predictor for ED[9], it could also act as a predictor for affective disorder[28,40]. Our results support further evidence related to the specificity of perfectionism on ED[6]. On the other hand, lower levels of socially prescribed perfectionism in the ED group were found in a previous study[41], which suggests that this dimension of perfectionism is not specific for ED. As we expected, eating psychopathology was highlighted in ED compared with the other control groups. Summarizing, owing to the previous

Table 3 Differences in biological variables and psychiatric background between case-control groups: ANOBAS study.

	ED [1] n=50	AD [2] n=40	AP [3] n=40	NP [4] n=50			ED vs AD	ED vs AP	ED vs NP	ED vs AD	ED vs AP	ED vs NP
					Friedman Test		Wilcoxon Test			Cohen's d		
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	χ^2	p	p	p	p	d	d	d
Cortisol	16.86 (4.53)	11.04 (4.15)	9.11 (3.92)	14.41 (4.79)	19.75	**p<.001**	**p<.001**	**p<.001**	**p=.007**	1.35	1.84	0.53
C3	83.13 (16.43)	100.77 (18.38)	94.1 (13.85)	98.13 (16.11)	15.13	**p<.002**	**p<.001**	**p<.001**	**p<.001**	1.03	0.72	0.93
C4	16.69 (4.53)	19.54 (7.55)	16.29 (5.23)	16.70 (3.90)	6.2	p=.11	-	-	-	0.48	0.06	0.0
Leptin	1864.01 (2430.13)	6394.81 (3693.46)	-	7498.77 (4903.02)	31.91	**p<.001**	**p<.001**	-	**p<.001**	1.5	-	1.47
	N (%)	N (%)	N (%)	N (%)	χ^2	p-valor	p-valor	p-valor	p-valor			
Current comorbidity Yes	18 (36)	12 (30)	5 (12.5)	4 (8)	15.17	**p=0.002**	p=.55	**p=.01**	**p<0.001**			
Psychiatric Background Yes	10 (20)	18 (45)	6 (15)	6 (12)	28.09	**p<0.001**	**p<0.001**	p=.28	p=.53			

Note. ED = Eating Disorders. AD = Affective Disorder. AP =Asthma Pathology. NP = Non-pathology group. M = Mean. SD = Standard Deviation. X2= Chi-square test. Leptin was not collected for the AP group. Significant values after Bonferroni correction are in bold.

evidence, it seems that the comparison with AD group will allow us to talk about specific psychological factors.

In addition, the results of our research empathised the strong similarities between ED and asthma families, as was expected. Similar levels were found between the groups in obsessive symptoms and depression of both parents, as well as similarities in family functioning. Related to this, both ED and AP studies have reported that the adequacy in the management of the disease is directly related to the quality of family functioning and also with family stress[42,43]. Regarding the similarities and differences between the groups, the AP group appears as the best suitable control group to investigate specific familial variables related to the onset of an ED.

On the other hand, the relation between correlates and the mix of psychological and biological variables is a good way to attend the complexity of ED aetiology. In line with previous findings, biological variables were also expected to stand out amongst the ED group when compared with the other groups. Cortisol and C3 levels were higher in the ED group, in concordance with the findings by Nova et al.[44], who found that the alteration of these factors was related to restrictive behaviours. On the other hand, leptin levels were lower amongst the ED group. Hebebrand et al.[18] have argued that high levels of leptin may work as a biomarker on the early detection of AN.

This study's methodology has some limitations. Firstly, the cross-sectional case-control design does not allow to infer causality; although, on the other hand, authors, such as Machado et al.[2] have argued that the replication of results can be a strategy to infer causality. Secondly, the measure of cortisol with one single blood sample in the morning and fasting is a limitation, since it may restrict the informative value, validity and interpretation of the cortisol value due to its circadian rhythm throughout the day. Thirdly, the majority of the ED group had an AN diagnosis. The study was offered to all the patients of the ED unit who met the inclusion criteria of having an evolution of less than 1 year, not had treatment and being between 12-17 years old. With a mean age of 14.64 the probability of finding AN is higher, but the course to other ED is not clear[45], so we decided to focus the study on ED in general. Fourthly, females with high socioeconomic status were predominant in this sample. Although it could be a limitation for the generalization of the results, female gender and high socioeconomic status is frequent in eating disorders[28,46], and matching for parental socioeconomic status reduces differences in family experiences related to availability of resources[22]. Lastly, only some of the variables proposed have been analysed in this manuscript, although they justified the suitability of the control groups. Lastly, evaluating the moment of the exposition to the correlate may control the precedence and allow quantifying the exposition of it.

CONCLUSIONS

In our knowledge, this is the first methodology proposal which assesses specific correlates including three control groups through a bio-psycho-family approach. The case-control design based on a rigorous sample matched by age and SES of the parents is appropriate to research correlates in EDs. The choice of suitable control groups is important in order to assess the specificity of the correlates. We found higher similarities between ED and affective disorder across psychological correlates and similarities between ED and asthma pathology on a familial level, so affective disorders and asthma pathology were suitable control groups to assess correlates in ED at the onset.

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CONFLICT OF INTEREST STATEMENT

The authors do not have any conflict of interest to declare.

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Appendix A: ANOBAS Assessment Protocol: psychological, familial and biological assessment.

Table A.1 Psychological assessment.

Variable, instrument	Original version.	Spanish validation.
Psychological variables		
Eating psychopathology Eating Attitudes Test (EAT-26) (47).	The EAT-26 is a 26-item test provides a good screening of eating disorders. A cut-off of 20 indicates disordered eating behaviours/ attitudes. It presented an internal consistency of .94.	Adapted by Gandarillas, Zorrilla and Sepúlveda (48). The internal consistency was .86 for the general population and .94 for the clinical sample.
Eating psychopathology Eating Disorder Inventory (EDI-II) (49).	The EDI-II is a 91-item questionnaire that evaluates attitudes and behaviors related to the diagnoses of ED through eleven subscales. The average internal consistency of the subscales ranged from .82 to .92.	The Spanish version adapted by Garner (50) had good internal consistency (Cronbach's alpha between .63 and .88 for the subscales and .92 for the total scale).
Body Dissatisfaction Body Shape Questionnaire (BSQ) (51)	The BSQ is a 34-item questionnaire employed to evaluate body dissatisfaction, fear of gaining weight and the desire to be thin. A cut-off of 115 was used, obtaining an alpha of .98.	Adapted by Raich, Mora, Soler, Avila, Clos and Zapater (52), with an internal consistency of .97.
Depression Level Children Depression Inventory (CDI) (53).	The CDI is a 27-item self-report designed to assess depression levels in children and adolescents. A cut-off point of 19 for teenage girls indicates risk of depression. Adequate internal consistency was found for this measure, obtaining a Cronbach's α of .86.	The Spanish version adapted by Del Barrio, Moreno-Rosset, & López-Martínez (54) had an internal consistency between .81 and .85.
Anxiety Level State-Trait Anxiety Inventory for Children (STAIC) (55).	The STAIC is a 40-item scale that evaluates the level of anxiety, differentiating between the state of anxiety and the anxiety trait. Alpha coefficients ranged from .85 for the State subscale and .84 for the Trait subscale.	The Spanish version was adapted by Seisdedos (56). Alpha coefficients ranged from .89 for the State subscale and .85 for the Trait subscale.
Obsesive Symptoms The Leyton Obsessional Inventory-Child Version (LOI-CV) (57).	The LOI-CV is a 20-item questionnaire that evaluates the presence or absence of obsessive concerns and behaviors in adolescents. It also evaluate the interference caused by each of the concerns or behaviors. Good internal consistency was obtained for this measure (Cronbach's $\alpha = .81$).	The Spanish version adapted by Serrano, Barrantes-Vidal, Domènech, Obiols and Subirá (58) had an internal consistency of .90.
Personality Traits The Junior Temperament and Character Inventory (JTCI) (59).	The JTCI is a 108-item questionnaire designed to evaluate personality based on the Cloninger personality model. The inventory presents good reliability, with values of between .44-.77 for the different scales.	The Spanish version by Pelaz, Bayón Pérez, Fernández-Liria and Rodríguez-Ramos (60) had an internal consistency between .42 and .76.
Perfectionism The Child-Adolescent Perfectionism Scale (CAPS) (61)	The CAPS is a 22-item questionnaire designed to evaluate perfectionism in adolescents. The CAPS establishes the difference between self-directed perfectionism and socially prescribed perfectionism and had an internal consistency of .85.	The Spanish version by Castro, Gila, Gual, Lahortiga, Saura and Toro (62) had an internal consistency of .89.
Vital Events Children's Life Events Inventory (63).	This scale considers 47 stressful life events and analyzes the impact and the moment when these events occur.	The Spanish version was adapted by Mardomingo and González (64).

Table A.2 **Familial assessment.**

Variable, instrument	Original version.	Spanish validation
Familial variables		
Depression Level Beck Depression Inventory (BDI) (65).	The BDI is a 21-item questionnaire that evaluates the level of depressive symptoms during the previous week. The cut-off points between 19-29 indicate a moderate level of depression and more than 30 indicates a severe level of depression. The inventory presents good reliability (Cronbach's α =.92).	The Spanish version adapted by Vázquez and Sanz (66) had high internal consistency (Cronbach's α =. 89).
Anxiety Level State and Trait Anxiety Inventory (STAI) (67).	The STAI is a 40-item scale designed to assess level of anxiety at the time of evaluation (anxiety-state) and the level of anxiety as a trait (anxiety-trait). The internal consistencies of these questionnaires are .86 and .86, respectively.	The Spanish version (68) had internal consistency between .83 and .92.
Obsesive Symptoms Obsessive-Compulsive Inventory-Revised (OCI-R; Foa et al., 2002).	The OCI-R is a 17-item scale designed to evaluate distress associated with obsessive symptoms through six subscales. The instrument presents good internal consistency, with an alpha coefficient of .90. The average internal consistency of the subscales ranged from .83 to .90.	The Spanish version adapted by Fullana, Tortella-Feliu, Caseras, Ansidon, Torrubia and Mataix-Cols (70) had an internal consistency of .86.
Personality Profile Temperament and Character Inventory-Revised (TCI-R) (71).	The TCI-R is a 240-item questionnaire designed to evaluate personality based on the personality model developed by Cloninger. The scale includes three dimensions related to character and four related to temperament. The internal consistency ranged from .60 to .87.	The Spanish version was adapted by Gutiérrez, Bayón, Valero, Labad, Cloninger and Fernández-Aranda (72) and had an internal consistency of .87.
Daughters' psychopathology, Child Behaviour Checklist (CBCL) (73).	The CBCL is a 113-item caregiver report designed to evaluate children' psychopathology. It provides eight psychopathological dimensions, which are common in children and adolescents. Internal consistency averages for the subscales of the CBCL ranged between .46 and .93.	The Spanish version (74) had adequate psychometric properties, with value ranged between .48 and .55.
Marital adjustment Short Marital-Adjustment and Prediction Test (75).	The questionnaire is a 15-item measure to assess marital success and adjustment. Questions are related to interests, occupation or finances. The internal consistency ranged between .72-.83.	The Spanish version was adapted by Carrobles (76), internal consistency is not tested.
Expressed emotion Family Questionnaire (FQ) (77).	The FQ is a 20-item instrument designed to measure expressed emotion in the family. It is divided into two subscales: criticism (CC) and emotional over-involvement (EOI). The internal consistency for the subscales was .92 and .80, respectively.	The Spanish version adapted by Sepúlveda et al. (78) had an internal consistency of .83 for the CC scale and .72 for the EOI scale.
Family functioning Family Adaptability and Cohesion Scale (FACES-II) (79).	The FACES-II is a 30-item self-report questionnaire designed to evaluate perceived family functioning. This scale offers three scores regarding cohesion, adaptation and the type of family functioning, with good internal consistency (Cronbach's α =.78-.87).	The Spanish version adapted by López Larrosa (80) had an internal consistency of .78 for cohesion scale and .70 for adaptability scale.
Coping mechanisms Coping Strategies (COPE-60) (81).	The COPE-60 is a 60-item questionnaire designed to assess coping mechanisms to solve problems. It includes 15 subscales related to 15 different coping styles. The internal consistency of the instrument ranged between .45-.92.	The Spanish version adapted by Crespo and Cruzado (82) had good psychometric properties.

Table A.3 Biological assessment

Type of Variable	Variables assessed	Procedure of the assessment
Biological variables	Blood samples were taken after an overnight fast. A Blood Sampling Questionnaire was previously answered in order to not perform the extraction if the adolescent presented an ongoing infection or had received a vaccination shot in the previous six weeks.	
Blood cell analysis	Red blood cell counts and indexes, haemoglobin, and white blood cells and differential (whole blood).	Automatic cell counter
Biochemical variables	Glucose, urea, uric acid, creatinine, pre-albumin, albumin, total protein, LDH, GOT, GPT, GGT, alkaline phosphatase, total bilirubin, total cholesterol and its fractions (HDL, LDL), triglycerides, Apo A1, Apo B, minerals (Ca, P, Na, K, Cl, Fe), transferrin, ferritin, vitamin B12, folic acid, retinol binding protein (RBP) in serum.	Colorimetric, nephelometric techniques and by electric potential using selective electrode (Na, K).
Immunological variables	Lymphocyte subsets: CD3, CD4, CD8+, CD19+, CD16+56+, CD45RO+, CD45RA+ . (1 mL blood (EDTA-K3) + 1mL preservative solution (Streck Cell PreservativeTM CE. Streck, USA))	Flow cytometry.
	Immunoglobulins: IgA, IgG, IgM, IgE in serum.	Nephelometry.
	Complement factors C3 and C4, C-reactive protein (CRP) in serum	Turbidimetry.
	Cytokine levels in serum: IL-1β, IL- 6, TNF-α, IL-2 in serum.	xMAP Technology for immunoassay of multiple analyses (Millipore).
Neuroendocrine variables	Free T4, T3, TSH, cortisol, insulin, FSH, LH, estrogens, testosterone, progesterone, prolactin, leptin, soluble leptin receptor, adiponectin, peptide YY (PYY) in serum.	RIA, ELISA and xMAP Technology for immunoassay of multiple analyses (Millipore).