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Executive functioning in anorexia nervosa patients and their unaffected relatives

Elisa Galimberti [a,b,*], Emma Fadda [a,b], Maria Cristina Cavallini [b], Riccardo Maria Martoni [b], Stefano Erzegovesi [b], Laura Bellodi [b,c]

[a] Experimental Neurology Institute, INSPE, Vita-Salute San Raffaele University, Italy
[b] Department of Clinical Neuroscience, San Raffaele Scientific Institute, Milan, Italy
[c] School of Psychology, Vita-Salute San Raffaele University, Milan, Italy

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ABSTRACT

Formal genetic studies suggested a substantial genetic influence for Anorexia Nervosa (AN) but currently results are inconsistent. The use of neurocognitive endophenotype approach may facilitate our understanding of the AN pathophysiology. We investigated decision-making, set-shifting and planning in AN patients ($n=29$) and their unaffected relatives ($n=29$) compared to healthy probands ($n=29$) and their relatives ($n=29$). The Iowa Gambling Task (IGT), the Tower of Hanoi (ToH) and the Wisconsin Sorting Card Test (WCST) were administered. Probands/relatives concordance rates and heritability index were also calculated. Impaired IGT and WCST performances were found in both AN probands and their relatives instead planning appeared to be preserved. IGT heritability index suggested the presence of genetic effects that influence this measure. No evidence for genetic effect was found for WCST. Results suggest the presence of a shared dysfunctional executive profile in women with AN and their unaffected relatives, characterized by deficient decision-making and set-shifting. Concordance analysis strongly suggests that these impairments aggregate in AN families supporting the hypothesis that they may constitute a AN biological markers. Decision-making impairment presents a moderate heritability, suggesting that decision-making may be a candidate endophenotype for AN.

1. Introduction

Anorexia nervosa (AN) is characterized by disordered eating behaviors where the patient's attitude toward weight and shape, as well as their perception of body shape, are disturbed (American Psychiatric Association, 2000). It has been suggested that the clinical phenomena seen in AN reflect executive functioning impairments (Frampton and Hutchinson, 2007). For example, AN patients continue to keep restrictive behaviors despite the serious risk for their health (Fairburn et al., 1999) and despite psychosocial consequences. These pathological eating behaviors reflect their preference to opt for choices that yield high immediate gains in spite of higher future losses and can be conceptualized in term of impairments in planning real-life strategies and decision-making abilities (Cavedini et al., 2004). Furthermore, AN patients are characterized by rigidity perfectionism, compulsive traits and they have a high need for control. These characteristics could be the results of set-shifting impairments (Tchanturia et al., 2012). An increasing number of neuropsychological studies have investigated the relationship between executive functions and disordered eating behaviors (Braun and Chouinard, 1992; Lauer, 2002; Duchesne et al., 2004; Southgate, et al., 2005; Tchanturia et al., 2005). Consistent findings have been found in set-shifting (Tchanturia, et al., 2002, 2004, 2012) (for review see Roberts, et al., 2007) and central coherence (Lopez et al., 2008). Poor set-shifting has been also found in unaffected sisters of women with AN, providing some evidence for this cognitive feature as a candidate endophenotype (Holliday and Tchanturia, 2005; Roberts, et al., 2010; Tenconi et al., 2010). Deficient decision-making has been reported in AN patients (Cavedini et al., 2004, 2006; Tchanturia et al., 2007), but recently Guillaume et al. (2010) found normal decision making in these patients, so this neuropsychological domain needs further elaboration. Unfortunately, even if AN pathological eating behaviors strongly suggest an impairment in planning strategies, only one study has investigated this function (Fowler et al., 2006) and no study has been conducted on their unaffected relatives.

At the present time, evidences regarding executive dysfunctions in AN patients remain unclear and many questions are unresolved. Particularly, there is uncertainty regarding the reversibility of some neurocognitive impairments after re-feeding.

* Corresponding author at: Department of Clinical Neuroscience, San Raffaele Hospital, Via Stamira D'Ancona 20, Milano, 20127, Italy.
Tel.: +39 0226433215; fax: +39 022643.
E-mail address: galimberti.elisa@hsr.it (E. Galimberti).

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Thus, whether executive deficits are state or trait related is a question still unresolved (Lindner et al., 2012; Cavedini et al., 2006).

The use of endophenotype approach may facilitate our understanding of AN executive functioning and pathophysiology. In the last few years, neurocognitive dysfunctions are considered among the most promising endophenotype candidates in many psychiatric disorders (Leboyer, 2003; Flint and Munafo, 2007; Bulik et al., 2007). This approach is an attractive strategy for the exploration of genetic predisposition because endophenotypes represent a means of dissecting the clinical phenotype into biological variable hypothetically more proximal to genetic effect (Leboyer, 2003; Flint and Munafo, 2007). This aspect is even more important taking into consideration that several decades of trying to discover causative genes in AN has, as yet, yielded disappointing results (Slof-Op't Landt et al., 2005; Scherag et al., 2010; Clarke et al., 2012).

Endophenotypes have to be heritable, co-segregating with a psychiatric clinical phenotype in the general population, state independent, and to be found in unaffected family members at a higher rate than in the general population (Flint and Munafo, 2007). Specific susceptibility gene variants may underlie endophenotypes, which in turn may predispose individuals to develop AN and related conditions. If a characteristic fulfils these criteria but is not proven to be heritable, it is termed a "biological marker" (Holliday and Tchanturia, 2005).

This study was designed to explore simultaneously planning, set-shifting and decision-making in AN patients and their unaffected first degree relatives. We used a test battery composed by the Tower of Hanoi (ToH), the Wisconsin Sorting Card Test (WCST) and the Iowa Gambling Task (IGT). We analyzed the proband/relative concordance rates for the cognitive performances. This is a relatively novel approach and could be useful to better understand the familiarity of the neurocognitive traits but it is not considerable as a measure of heritability since all family members lived in the same household and share genes as well as environments. To better understand if these executive functions could be considered a candidate endophenotype for AN, we calculated heritability indices (h2) using parent–offspring regression model.

Finally, instead of looking at each neurocognitive performance separately, we analyzed the combination of performances together in order to define complex neurocognitive profiles. Results from this study might provide further supporting evidence for the possible qualification of planning, decision-making and set-shifting, as a candidate endophenotype for AN.

2. Methods

2.1. Subjects

The study sample consisted of 116 subjects: 29 pairs of AN probands and their unaffected first-degree relatives and 29 pairs of healthy comparison probands and their unaffected first-degree relatives. All participants were female. AN relatives group consisted of 18 mothers and 11 sisters. In HC relatives group there were 15 mothers and 14 sisters.

AN probands were recruited consecutively from a clinical population referring to the Department of Neuropsychiatric Sciences, San Raffaele Hospital, Milan. Inclusion criteria for AN probands were: (a) the willingness to participate and to involve their relatives in the study; (b) diagnosis of Anorexia Nervosa according to DSM-IV-TR (American Psychiatric Association, 2000); (c) absence of lifetime Axis I diagnosis; (d) absence of mental retardation and/or neurological illness and/or brain injury or trauma; (e) history of drug or alcohol abuse; (f) age between 18 and 65 years. For 1 year, all patients receiving treatment within the Units for the treatment of eating disorders were asked to participate in the study. During a clinical interview, a senior psychiatric verified if patients fulfilled all inclusion criteria and administered the Mini-International Neuropsychiatric Interview (MINI-DIS) (Sheehan et al., 1998), a well-validated screening instrument for Axis I disorders. If patients satisfied study's inclusion criteria, with their permission, the relative was contacted and invited to take part in the study. If the patient had more than one sister, the sister closest in age was recruited. In order to participate in the study, AN relatives had to satisfy the same inclusion criteria of their probands (excepted for AN diagnosis). Fourteen patients satisfied criteria for AN restricting subtype (AN-Re) and 15 patients for binge-purge subtype (AN-Be). All patients were unmedicated.

HC probands of normal weight with no history of eating disorders and their relatives were recruited in the local community. Exclusion criteria were: (a) lifetime Axis I diagnosis according to DSM-IV-TR (American Psychiatric Association, 2000); (b) history of mental retardation; (c) neurological illness; (d) brain injury or trauma; (e) history of drug or alcohol abuse; and (f) age between 18 and 65 years. During an interview, a senior psychiatric verified if HC probands and their relatives fulfilled all inclusion/exclusion criteria and administered MINI-DIS (Sheehan et al., 1998).

This study was designed in accordance with the Declaration of Helsinki and approved by the Milan Area Health Authority Ethics Committee. Written informed consent was obtained from all participants after the procedure has been fully explained.

2.2. Assessment

In AN probands, severity of illness was assessed using the Yale–Brown Cornell Scale for Eating Disorders (Mazure et al., 1994) and the Body Mass Index (BMI), expressed as kg/m^2, was measured for each patient. Onset and duration of illness were also collected.

The following neuropsychological validated tasks were administered: (1) the Iowa Gambling Task (IGT) (Bechara et al.,1994), that assesses decision-making, the ability to acquire a preference through reward and punishment as represented by money gains and losses; (2) the Tower of Hanoi (ToH) (Shallice, 1982), that assesses planning, the ability to achieve a goal through a series of intermediate steps; and (3) the Wisconsin Card Sorting Test (WCST) (Bergh, 1948), that explores set-shifting, the ability to shift to a different thought or action according to the situation's context. A full description of the administered tasks is provided in a previous publication (Cavedini et al., 2010).

2.3. Procedure

If participants satisfied study's inclusion criteria, executive functions were then assessed. The neuropsychological tasks were administered by a trained psychologist in a single session and in a randomized tasks sequence. The complete testing session never lasted more than 90 min and all participants completed the tests without problems of cooperation or fatigue.

2.4. Statistical analysis

Data were collected and analyzed using the Statistica 6.0 (StatSoft Inc, Tulsa, Oklahoma). The four groups were compared on demographic and neuropsychological variables by using an analysis of variance (ANOVA).

Analysis of data from relatives pairs need to account for the fact that measures are not independent. Because members of the same family share a number of characteristic, observations from the same family pair might be positively correlated. We deal with this by using ANOVA to compare exclusively AN vs. HC probands, AN vs. HC relatives and AN relatives vs. HC probands. Since age might potentially influence neuropsychological performances, this variable was included as covariate to control for this potential confounder factor.

The t-test for dependent group was used to carry out comparisons between probands and relatives in both groups, because of the lack of independence between family members.

In order to exclude possible differences in neuropsychological performances, using the same statistical model, we compared mothers vs. sisters, probands vs. sisters and probands vs. mothers performances in both clinical and healthy groups.

In a subsidiary analysis, to assess the possible influence of clinical subtype on neuropsychological performances, the ANOVA was used to compare AN patients who met criteria for Restrictive subtype and those belonging to Binge-Purge subtype.

Spearman's correlation coefficient was used to determine relationships between clinical variables and neuropsychological test scores.

In a subsidiary analysis, heritability indices (h2) for neuropsychological tasks were calculated. Since there was no difference in neuropsychological performances between sisters and mothers in both HC-R and AN-R groups (see results Section 3) and considering the relatively small sample size, we used parent–offspring regression model to estimate h2 (Falconer and Mackay, 1996; Lynch and Walsh, 1998). Using this model, the slope of the regression line approximates the heritability of the neurocognitive traits when offspring values are regressed against the value in the mother.

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Finally, probands/relatives good/bad performances concordance rates were analyzed. To differentiate between good and bad performance in each test, a cut-off point was estimated using Separate Receiver Operating Characteristic Curves (ROC). ROC curves were plotted for each test score vs. MINI-DIS AN diagnosis from three large independent samples of AN patients and one sample of HC subjects. It should be noted that there is a partial overlapping in the HC sample used to calculate ROC curves and the HC sample used in Cavedini et al. (2010). Moreover, there is a partial overlapping between our AN sample used for to calculate ROC curves and the AN sample of Cavedini et al. (2006).

Epidemiological variables of these samples and those included in the study were compared, and no significant differences were found (see Appendix B). A cut-off point with the best balance between sensitivity and specificity indices was used. The area under the curve was used as an overall indicator of the discriminant validity of the instrument (see Appendix A). Four different profiles were identified according to proband/relative performance (Good/Good, Good/Bad, Bad/Good, and Bad/Bad). The Pearson χ^2 was used to compare the frequency of the four profiles between clinical and healthy groups.

Finally, it should be noted that regarding WCST performance we have decided to analyze the concordance rate and heritability index only for the percentage of perseverative errors. Firstly, this outcome is a measurement of perseveration and flexibility (Tchanturia et al., 2012) and it is used traditionally as a measure of set-shifting ability (Roberts et al., 2007), which may be linked to compulsive traits, rigidity and perfectionism that are considered "core" traits of AN. Secondly, a recent study has shown that people with a past history of AN showed better WCST performance than actively ill participants; however preseverative errors were significantly impaired compared to HC participants (Tchanturia et al., 2012). These data suggest that the number of perseverative errors could be a promising candidate endophenotype of AN.

3. Results

3.1. Demographic and clinical characteristics

Means ± Standard Deviation for demographic and clinical characteristics are shown in Table 1. AN and HC probands were equivalent in age ($F(1,56)=3.09$; $P=0.08$) and educational level ($F(1,56)=1.31$; $P=0.25$). The same results were obtained comparing AN and HC relatives [Age: $F(1,56)=0.01$, $P=0.89$); Educational level: ($F(1,55)=0.59$, $P=0.44$)]. We found differences for age but not for education comparing AN and HC probands to their respective relatives [AN: Age ($F(1,56)=50.06$; $P=0.0000$); Education: ($F(1,56)=0.29$; $P=0.58$)] [HC: Age ($F(1,56)=50.06$; $P=0.0000$); Education: ($F(1,56)=0.03$; $P=0.84$)]. Furthermore, restrictive and binge-purge patients did not differ on age [(AN-Re: 25.1 ± 7.02; AN-Be: 22.5 ± 6.63; ($F(1,27)=1.00$; $P=0.32$)], onset [(AN-Re: 18.58 ± 5.56; AN-Be: 17.8 ± 3.79; ($F(1,27)=0.19$; $P=0.66$)] and duration of illness [(AN-Re:7 .35 ± 5.7; AN-Be: 4 ± 3.38; ($F(1,27)=3.29$; $P=0.08$)]. Clinical subtypes were also equivalent in severity of illness assessed by Y-Cornell [(Preoccupation Score: AN-Re: 12.33 ± 3.65; AN-Be: 14 ± 2.13; ($F(1,27)=1.95$; $P=0.17$); Rituals Score: AN-Re: 11.53 ± 3.77; AN-Be: 13 ± 3.49; ($F(1,27)=1.07$; $P=0.30$)] and BMI.

3.2. Neuropsychological assessment

Means and ± Standard Deviation for neuropsychological tasks performances are shown in Table 2. Performing IGT, HC probands choose more frequently from the advantageous decks than AN probands ($F(1,56)=6.79$; $P=0.01$). AN relatives choose less cards from advantageous decks compared to HC relatives ($F(1,56)=24.20$; $P=0.00001$) and HC probands ($F(1,56)=21.8$; $P=0.00002$). No difference was found comparing AN and HC probands to their respective relatives in the number of advantageous choices [(AN: $t=-1.81$; $p=0.15$; HC: $t=-0.48$; $P=0.62$)].

In both relative groups, mothers and sisters were equivalent in the number of advantageous choices made during the task execution [(HC: ($t=-1.8$; $P=0.09$); AN: ($t=-1.51$; $P=0.16$)]. Finally no difference was found comparing the performances of AN-Re and AN-Be subtype ($F(1.27)=0.34$; $P=0.56$).

On the ToH task, the number of moves in excess of the mathematical minimum was higher in AN probands compared

Table 1
Demographic and clinical characteristics of samples.

	AN(P) $N=29$ ($M \pm$ S.D.)	AN(R) $N=29$ ($M \pm$ S.D.)	HC(P) $N=29$ ($M \pm$ S.D.)	HC(R) $N=29$ ($M \pm$ S.D.)
Age (years)	24.10 ± 6.8	43.79 ± 12.9	28.62 ± 11,9	43.31 ± 13.6
Education (years)	13.37 ± 3.5	13.96 ± 4.6	14.34 ± 2.8	14.17 ± 3.7
Y-Cornell preoccupation score	13.07 ± 3.13	/	/	/
Y-Cornell rituals score	12.18 ± 3.65	/	/	/
BMI	16.21 ± 4.02	/	/	/
Age of onset	18.13 ± 4.53	/	/	/
Duration of illness(years)	5.95 ± 5.09	/	/	/
Gender (f/m)	29/0	29/0	29/0	29/0

AN, Anorexia Nervosa; HC, Healthy comparisons subjects; P, Probands; R, Relatives; BMI, Body Mass Index.

Table 2
Quantitative performance in neurocognitive tasks.

Task	AN Probands ($N=29$) ($M \pm$ S.D.)	AN Mothers ($N=18$) ($M \pm$ S.D.)	AN Sisters ($N=11$) ($M \pm$ S.D.)	HC Probands ($N=29$) ($M \pm$ S.D.)	HC Mothers ($N=15$) ($M \pm$ S.D.)	HC Sisters ($N=14$) ($M \pm$ S.D.)
IGT[a,b]	53.7 ± 11.8	47.09 ± 8.6	53.36 ± 9.02	63.6 ± 11.5	58.8 ± 10.6	67 ± 11.82
ToH	5.03 ± 4.3	6.36 ± 6.6	3.72 ± 3.9	2.5 ± 2.2	2.07 ± 1.81	1.71 ± 1.38
WCST perseverative errors[a,b]	7.79 ± 6.2	13.52 ± 10.5	13.67 ± 11.63	4.47 ± 2.5	4.51 ± 2.32	4.83 ± 2.72
WCST total errors[a,b]	16.93 ± 14.4	24.33 ± 15.52	22 ± 15.18	9.55 ± 4.08	12.13 ± 5.98	9.35 ± 4.01

IGT, Iowa Gambling Task (advantageous deck selection), high score indicate better performance respect to low score; ToH, Tower of Hanoi (number of moves in excess), low score indicate better performance respect to high score; WCST, Wisconsin Sorting Card Test (% of perseverative errors), high score indicate better performance respect to low score.

[a] HC Probands vs. AN Probands: $p < 0.05$.
[b] HC Relatives vs. AN Relatives: $p < 0.05$.

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to HC probands, but these differences did not reach significance when age was applied as covariate in the ANOVA model ($F(1,56)=0.4$; $P=0.83$). The same result was obtained comparing AN relatives to HC relatives ($F(1,56)=0.34$; $P=0.55$) and AN relatives to HC probands ($F(1,56)=2.42$; $P=0.12$). Furthermore, compared to the respective relatives, both AN and HC probands were equivalent in the ToH performances [(AN: $t=-0.33$; $P=0.74$; HC: $t=-1.04$; $P=0.30$)]. In both groups, mothers and sisters employed the same number of moves to complete the ToH [(HC:($t=-0.75$; $P=0.46$); AN:($t=1.01$; $P=0.33$)]. No difference was found comparing AN-Re and AN-Be subtypes on ToH ($F(1,27)=0.43$; $P=0.51$).

Regarding WCST, we analyzed both the total number of errors and the percentage of perseverative errors. Regarding perseverative errors, AN probands showed a high percentage than HC probands ($F(1,56)=4.35$, $P=0.04$). The same pattern was observed comparing AN relatives to HC relatives ($F(1,56)=5.74$; $P=0.03$) and AN relatives to HC probands ($F(1,56)=4.92$; $P=0.03$). No difference in the percentage of perseverative errors was found comparing AN and HC probands to their respective relatives [(AN: $t=-1.87$; $P=0.09$; HC: $t=-0.37$; $P=0.71$)]. Finally, mothers and sisters made the same number of errors to complete the task in both clinical and healthy groups [(HC: ($t=-0.315$; $P=0.75$); AN: ($t=-0.02$; $P=0.97$)]. No difference was found between AN-Re and AN-Be subtype in the WCST ($F(1,27)=0.003$; $P=0.95$). The same pattern of results was found analyzing the total number of WCST errors. AN probands committed a higher number of errors compared to HC probands ($F(1,27)=6.97$; $P=0.01$); furthermore, there was a significant difference comparing HC and AN relatives ($F(1,27)=18.04$; $P=0.000$). Comparing AN and HC probands to their respective relatives no difference has been found in the total number of errors [(AN: $t=-1.67$; $P=0.09$; HC: $t=-1.00$; $P=0.31$)]. Finally, mothers and sisters made the same number of errors in both clinical and healthy groups [(AN: ($t=-0.39$; $P=0.69$); HC: ($t=-1.45$; $P=0.15$)].

Severity, assessed by Yale-Brown Cornell Scale scores, BMI, onset and duration of illness did not correlate with any neurocognitive performances (all Pearson correlation coefficients below 0.20). Furthermore, we performed correlation analysis between age and neuropsychological indexes and none of them resulted significantly.

Based on presented data, heritability indices (h2) were estimated by comparing mother and offspring neurocognitive performances in IGT and WCST, in both clinical and healthy groups, using parent–offspring regression analysis model. Heritability indexes for the two neurocognitive tasks in each group are shown in Table 4.

3.3. Concordance of probands and relatives with regard to neuropsychological performance

Proband/relative concordance with regard to task performance was analyzed for each task. Four different profiles were identified according to proband/relative performance: Good/Good, Good/Bad, Bad/Good, and Bad/Bad. Results are shown in Table 3. Concordance profile rates were not calculated for Toh, because all groups showed equivalent performances on this task.

Comparing percentages of different concordance profiles of clinical and healthy groups, an overall significant difference was found in the IGT ($P=0.007$) and in the WCST ($P=0.006$). Furthermore, in the two neurocognitive tasks, there was a significantly higher percentage of bad/bad concordance in clinical than in the healthy groups (IGT: $P=0.03$; WCST: $P=0.03$) and a significantly higher percentage of Good/Good concordance in HC sample than in the clinical one (IGT: $P=0.0006$; WCST: $P=0.0006$).

Table 3
IGT Concordance Profiles between Probands and relatives.

	G/G (%)	**G/B** (%)	**B/G** (%)	**B/B** (%)
IGT				
HC ($n=29$)	(21) 72.41	(4) 13.79	(2) 6.90	(2) 6.90
AN ($n=29$)	(8) 27.59	(9) 31.03	(4) 13.79	(8) 27.59
p-level	$P=0.0006$	n.s.	n.s.	$P=0.03$
WCST				
HC ($n=29$)	(21) 72.41	(3) 10.34	(3) 10.34	(2) 6.90
AN ($n=29$)	(8) 27.59	(8) 27.59	(5) 17.24	(8) 27.59
p-level	$P=0.0006$	n.s.	n.s.	$P=0.03$

G/G good performance in proband/ good performance in relatives; G/B, good performance in probands/bad performance in probands; B/G bad performance in probands/good performance in relative; B/B, bad performance in probands/ bad performance in relatives; HC, Healthy comparison subjects; AN, Anorexia Nervosa; IGT, Iowa Gambling Task; WCST, Wisconsin Card Sorting Test.

Table 4
Heritability Indices (h2) for Iowa Gambling Task and Wisconsin Sorting Card Test.

Task	AN h2	HC h2
IGT (Advantageous deck selection)	0.42	0.40
WCST (% of perseverative errors)	0.20	0.32

h2, heritability index; IGT, Iowa Gambling Task; WCST, Wisconsin Sorting Card Test; AN, Anorexia Nervosa; HC, Healthy comparison subjects.

In order to define a complex neurocognitive profile characterizing women with AN and their relatives we analyzed the combination of bad/good performance on all three tasks instead of looking at each separately. In our sample, half of the AN patients (48.26%) and their relatives showed a bad performance at two tests simultaneously compared to the 7.14% and the 9.9% in HC probands and their relatives respectively. The percentage of the nine possible neurocognitive profiles in all groups is shown in Appendix C.

4. Discussion

The aim of this study was to examine decision-making, planning and set-shifting in AN patients and their unaffected first-degree relatives. The Iowa Gambling Task (IGT), the Tower of Hanoi (ToH) and the Wisconsin Sorting Card Test (WCST) have been administered to all participants.

Results showed that both AN patients and their unaffected relatives performed significantly poorer than HC and their relatives at IGT and WCST, suggesting that deficient decision-making and set-shifting characterized AN patients and were shared by their healthy relatives. Planning abilities, assessed by the ToH, appeared to be preserved in both AN patients and their unaffected relatives. The set-shifting difficulties found in our patients and their relatives are largely consistent with previous studies that assessed this function in AN (Tchanturia et al.,2012) (for review see, Roberts et al., 2007), and with previous studies that found poor set-shifting in unaffected sisters of women with AN, providing some evidence for this cognitive feature as a candidate endophenotype (Holliday and Tchanturia, 2005; Roberts et al., 2010; Tenconi et al., 2010).

The available data regarding decision-making in AN patients are still contradictory. Some studies have shown impaired decision-making in AN patients assessed by Gambling Task (Cavedini et al., 2004; Cavedini et al., 2006) and Iowa Gambling Task (Tchanturia, et al., 2007; Brogan et al., 2010; Abbate-Daga

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et al., 2011), while other studies did not find significant difference in IGT performance between AN patients and HC (Bosanac et al., 2007; Guillaume et al., 2010; Lindner et al., 2012). Guillaume et al. (2010) suggested that the decision-making impairments that have been found in previous works could be explained by potential confounding factors such as a high level of depression or medication (Guillaume et al., 2010). The possible influence of medication, particularly SSRI, is still unclear. Some evidences suggested that serotonin system is involved in decision making (Jollant et al., 2007a, 2007b) suggesting that SSRI may influence this neurocognitive function. However, Cavedini et al. (2004) analyzing AN patients free from any medication have found impaired decision-making in these patients. Furthermore, Tchanturia et al. (2007), did not find any influence of SSRI on IGT performance in AN patients. In our sample patients are unmedicated, so, results suggest that AN patients are characterized by decision-making deficits independently from the possible influence of medication.

As suggested by Guillaume et al. (2010), "the impact of depression on decision making is unclear as some authors have found that depressive symptoms can affect decision-making performance, whereas others have argued that decision making is not influenced by current depressive episodes". For this reason, we decided to exclude patients with comorbid Axis I disorders. It should be noted that we did not assess possible subclinical symptoms of depression that could have influenced the task performance. This is a limitation of the study and the findings should consider it as a caveat.

No previous studies have assessed decision-making in AN relatives, so this is the first evidence that suggests the presence of deficient decision-making in mothers and sisters of patients with AN. Although decision making has not been studied in relatives of eating disorders, some studies have reported decision-making alterations in unaffected relatives of alcoholics (Lovallo et al., 2006) and in unaffected relatives of patients with obsessive compulsive disorder (Cavedini et al., 2010).

We cannot exclude the absence of decision-making and set-shifting deficits before the onset of the disorder, so we compared neurocognitive performances between patients presenting low (12.5) and high BMI (17) and we did not find differences in any task, suggesting that such deficits are independent from weight status. Moreover, correlational analysis performed on severity of illness scores, decision-making and set-shifting performances also suggested the trait nature of these dysfunctions.

The trait nature of set-shifting impairments is in line with a recent work evaluating the relationship between severity of illness and cognitive flexibility. Tchanturia et al. (2011) failed to find correlation between severity of illness, measured by length of illness, and the lack of flexibility, in people with AN. The same authors analyzed a large dataset evidencing that recovered AN participants showed a better performance than currently ill participants; however, the number of perseverative errors was higher than for HC participants (Tchanturia et al., 2012).

Regarding decision-making four studies assessed decision-making in weight restored or recovered AN patients (Bosonac et al., 2007; Tchanturia et al., 2007; Jollant et al., 2007a, 2007b; Lindner et al., 2012) showing no significant difference in IGT performance comparing recovered AN patients and the healthy control. Our results seem to contradict these evidences. One possible explanation could be the experimental design used in the previous literature. In fact, as suggested by Lindner et al. (2012) the cross-sectional design used in these studies did not explore whether the better decision-making performance of the recovered or weight restored AN patients was present even before treatment. Only one study has assessed decision-making before and after treatment (Cavedini et al., 2006) and results revealed that impairment in decision-making was stable over time and independent of the physical and clinical changes after treatment. Our correlational analysis is in line with this hypothesis. Furthermore, the presence of decision-making impairments in AN relatives suggests that these impairments may both pre-exist and maintain AN development, rather than simply being a consequence of the illness itself.

Unexpectedly, we did not found abnormal planning abilities in AN patients assessed using the Tower of Hanoi. It should be noted that, even if there is no significant difference between AN and HC probands, descriptively AN patients and their relatives required a higher number of moves to complete the task respect to HC and their relatives. It is possible that the lack of significant difference between AN and HC could be explained by the sample size. To our debate, only one study has investigated this complex function in AN (Fowler et al., 2006). Using the Stocking of Cambridge Task, Fowler et al. (2006) showed significantly but moderate planning impairment in AN patients. Furthermore, these authors found that AN patients solved significantly fewer planning problems in the minimum number of moves than the control group but evidenced that the impairment is attributable to a slight increase in the number of moves required to solve the problem at the high level of difficulty. It should be noted that, although the clinical phenomena associated with AN suggested that they may have impaired planning abilities, this neurocognitive function has received little attention in previous studies. Further research, should deeply examine planning abilities in patients with AN and other eating disorders using large samples and multiple planning tasks.

Regarding concordance analysis, we found a significantly higher concordance rate for bad/bad performance in the IGT and WCST in the clinical group compared to healthy group. Furthermore, a higher concordance rate for good/good performance in these tests was found in healthy group, suggesting that decision-making and set-shifting performances aggregate in these families. These data suggest that, even though relatives do not manifest symptomatology fulfilling DSM-IV criteria (American Psychiatric Association, 2000) for AN diagnosis, alterations in their decision-making and set-shifting abilities may represent a vulnerability substrate that, in the presence of additional but still unknown risk factors, leads to the manifestation of AN. Proband/relative concordance analysis is a relatively new approach and could be useful to better understand the familiarity of the neurocognitive performances but could not be considered a measure of heritability since all family members lived in the same house, hold and shared genes as well as environments.

In order to understand if the evaluated neurocognitive performances could be considered a candidate AN endophenotypes, we estimated heritability indices (h2) for IGT and WCST performances using parent–offspring regression model. In fact, to consider a trait an endophenotype, it must be heritable and be closer to the genes responsible for the illness (Leboyer, 2003; Flint and Munafo, 2007). IGT heritability indices (AN: $h2=0.40$; HC: $h2=0.42$) suggested a likely presence of genetic effects that influence this measure, supporting the hypothesis that decision-making could be a potential neurocognitive endophenotype for AN. This suggest that impairments in decision-making may both pre-exist and maintain AN development, rather than simply being a consequence of the illness itself. In accordance with our results, many studies have showed influence of genetic variant on IGT's performance (Jollant et al., 2007a, 2007b), but unfortunately, no previous studies calculated heritability IGT indices, so, further researches using larger sample are needed.

Regarding set-shifting, WCST heritability indices (AN: $h2=0.20$; HC: h2: 0.32) suggested no evidence of genetic influence for this task. Previous research on the heritability of WCST performance is

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sparse, and mixed. Some evidences showed influence of genetic variants on WCST's performance (Liao et al., 2009), whereas other evidences contradict this hypothesis. For example, Taylor and colleagues administered WCST to 80 monozygotic and 29 dizygotic twin pairs screened for absence of neurological disease and head injury. Results showed little evidence of genetic influence, suggesting that it might not satisfy one of the criteria for an endophenotype (Taylor, 2007). Recently, Anokhin and colleagues examined heritability of performance on WCST in a longitudinal sample of adolescent twins. The results suggested increasing influence of familial factors on this performance, as well as gender differences in the relative role of genetic and environmental factors (Anokhin et al., 2010).

In the last years, set shifting has been considered as the most promising candidate endophenotype for AN (Holliday and Tchanturia, 2005; Roberts et al., 2010; Tenconi et al., 2010). Our results suggest that poor set-shifting assessed using WCST could be considered a possible "biological marker" instead of a candidate endophenotype of this disorder. In our opinion, one possible explanation of our results is that WCST is not a very sensitive task for assessing set-shifting. This may explain the contradiction between the lack of results found here and the general idea suggesting set-shifting as a promising endophenotype in AN. Furthermore, it should be noted that we analyze only the percentage of perseverative errors during the WCST performance and we did not analyze other WCST outcome measures. It is possible that other WCST outcome measures meet all endophenotype criteria. Further researches assessing heritability of all WCST outcome measure and using other neurocognitive task assessing set-shifting abilities in AN patients and their relatives are needed.

Finally, we analyzed the combination of the three neurocognitive performances instead of looking at each of them separately, in order to define complex neurocognitive profiles that could characterize AN probands and their relatives. In our sample, half of the AN patients (48.26%) and their relatives showed a bad performance at two tests simultaneously compared to the 7.14% and the 9.9% in HC probands and their relatives respectively. Data suggest that both AN patients and their relatives showed at least two deficient executive functions contemporary but, at the present time, it is not possible to define a specific neurocognitive profile.

The potential usefulness of creating neuropsychological profiles that include a range of abilities is also suggested by Lask and colleagues that are developing a standardized battery of tests to assess neurocognitive functioning in AN (Rose et al., 2011; Stedal et al., 2012; Rose et al., 2012).

Authors have developed this test battery in order to ensure a consistent methodology when researching on neuropsychological functioning in AN patients. In fact, one of the problems in this research area is the lack of methodological consistency between studies (Rose et al., 2011). In our opinion, the developments of standardized methodology to assess AN patients are very important, but it is also important to focalize our attention on possible relationships between different deficient neurocognitive domains. In fact, study of executive functions singularly is a simple approach that does not account for the complexity of the neurocognitve functioning. The creation of complex profiles containing a range of functions and the study of their own relationships could be more closely related to the AN brain functioning complexity.

Furthermore, this approach could account for the problem of neuropsychological impairment specificity in psychiatric disorders. In fact, executive functions deficits were found across psychiatric conditions, showing that they are not specific to the eating disorders field (Jurado and Rosselli, 2007; Royall et al., 2002). It should be noted that we have decided to recruit only patients without comorbid Axis I disorders, as well as AN relatives did not show any Axis I lifetime diagnosis. It might be argued that our AN sample could be not considered representative of the general population and our decision could be considered a study's limitation. In our opinion, this methodological decision could be also considered a study's strength. In fact, considering the problem of neuropsychological impairment specificity in psychiatric disorders, our approach permits to determine whether the pattern of executive impairments reported in our sample is specific to AN and is not a feature of other axis I disorders.

This study has some limitations. The sample is small and the findings should have this as a caveat. The sample size could represent a study's limitation particularly in the interpretation of heritability index, so, replication with a larger sample is needed to substantiate our finding regarding the possible influence of genetic effects on IGT and WCST measures. We did not consider personality disorders comorbidity as a possible confounding variable. Finally, we decided to recruit AN mothers and not only sisters because mothers should have less residual risk of developing the illness.

In conclusion, results suggest the presence of a shared dysfunctional executive profile in women with AN and their unaffected relatives, characterized by deficient decision-making and set-shifting. Data from concordance analysis strongly suggest that these executive impairments aggregate in these families and are in agreement with the hypothesis that the pattern of executive functions observed may constitute a biological markers in AN. Particularly, decision-making impairment, seems to present a moderate heritability, providing support for the possibility that deficient decision-making may be a part of the endophenotype in this disorder.

Finally, the creation of complex neurocognitive profiles can contribute to improve our knowledge about the executive functioning in women with AN and thus help to distinguish this disorder to other psychiatric disorders that also exhibit executive dysfunctions.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.psychres.2012.10.001.

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