The 2D:4D ratio and its relationship with other androgenisation parameters in parents of individuals with autism spectrum disorders

Ángel Romero-Martínez, Sara de Andrés-García, Patricia Sariñana-González, M.V. Sanchis-Calatayud, Juan M. Roa, Esperanza González-Bono and L. Moya-Albiol*

Department of Psychobiology. Faculty of Psychology. University of Valencia, Spain

Título: La ratio interdigital D2:D4 y su relación con otros indicadores de androgenización en progenitores de personas con trastornos del espectro autista.
Resumen: La ratio D2:D4 es el cociente entre la longitud de los dedos índice y anular e indicador indirecto de androgenización. A mayor testosterona prenatal,incrementa la probabilidad de desarrollar una menor ratio D2:D4. Se ha sugerido que personas con trastornos del espectro autista (TEA) y sus progenitores podrían tener cerebros altamente androgenizados, por lo que se ha utilizado como marcador de tal idiosincrasia. El objetivo principal es analizar si los progenitores de personas con TEA difieren de la población general en diversos parámetros de androgenización, principalmente la ratio D2:D4. La muestra estuvo compuesta por 43 progenitores de personas con TEA y 42 controles, de los que se midieron la ratio D2:D4, cuestionarios rasgo y niveles de testosterona y cortisol en saliva. Aunque no se encontraron diferencias por grupo en la ratio D2:D4, la ratio de la mano izquierda de progenitores de personas con TEA mostró mayor capacidad predictiva para explicar los cocientes de empatía y autismo, conductas cooperativas y niveles de cortisol. Además, en los progenitores varones predijo la gravedad de los hijos. Los resultados obtenidos indican que la ratio D2:D4 podría ser empleada, junto a otros parámetros, como indicador de la probabilidad de desarrollar rasgos autistas en los descendientes.
Palabras clave: Androgenización; cortisol; ratio D2:D4; trastornos del espectro autista; testosterona.

Abstract: The 2D:4D ratio is the quotient between the index and ring finger lengths and is a non-direct indicator of androgenisation. If prenatal testosterone levels in the amniotic fluid are high then the probability increases of developing lower ratio values. It has been suggested that people with autism spectrum disorders (ASD) and their parents may have highly androgenised brains, and for this reason the 2D:4D ratio is used as a marker of such idiosyncrasies. This study aims to analyse if parents of people with ASD differ from the general population in several parameters of androgenisation related to the 2D:4D ratio. The sample was composed of 43 parents of offspring with ASD and 42 controls who had the 2D:4D ratio measured, answered several trait questionnaires, and had their testosterone and cortisol levels measured. Although there were no differences between groups in the 2D:4D ratio, the left hand of the ASD parents showed greater predictive ability to explain empathy and autism quotients, cooperative behaviour, and cortisol levels. In addition, the severity of the symptoms of their offspring was predicted only with male parents. The results indicate that the 2D:4D ratio could be used together with other parameters as an indicator of the likelihood of developing autistic traits in offspring.
Key words: androgenisation; cortisol; 2D:4D ratio; autism spectrum disorders; testosterone.

Introduction

The 2D:4D ratio or quotient between the index and ring finger lengths is considered a non-direct indicator of androgenisation (Schneider, Pickel & Stalla, 2006). Accordingly, the greater the exposure and sensitivity to prenatal testosterone and corresponding reductions in oestrogens, the greater is the likelihood of developing a lower ratio (Manning, Bundred, Newton & Flanagan, 2003). Therefore, men will show, in general, lower values for this parameter than women (Breedlove, 2010). Additionally, the stability of the ratio over time in humans from two years of age has been proven, and there are no major variations during puberty (Knickmeyer & Baron-Cohen, 2006).

In humans, the 2D:4D ratio has been associated with health, cognitive profiles, physiological variables, personality patterns, and with the activator effects of hormones in adulthood. However, the results that relate these variables have been conducted, in general, with different samples and in separate studies. This fact makes it difficult to extract integral conclusions when explaining which role this indicator plays as a predictor of possible interactions between androgenisation, cognition, and health.

* **Dirección para correspondencia [Correspondence address]:**
Luis Moya-Albiol, Departament of Psychobiology. Faculty of Psychology. University of Valencia. Avenida Blasco Ibañez, 21. 46010, Valencia (Spain). E-mail: Luis.Moya@uv.es

In terms of cognitive patterns, men often have more interest in objects and the laws underlying reality than for people and so they are more systematic (Baron-Cohen, 2002). This cognitive style is named 'S type', whereas an excessive systematising has been named 'S extreme', and is related to people affected by autism spectrum disorder (ASD). According to empathy and systematising theory, this cognitive style represents one of the endpoints of a continuum where people who are excessively empathetic will be situated at a far end called 'extreme E' (Baron-Cohen, 2010a). These cognitive styles have been related with the 2D:4D ratio, which means low ratio-values are associated with high values in systematising and less in empathy (Von-Horn, Bäckman, Davidsson & Hansen, 2010). Furthermore, women with lower 2D:4D ratios, or with more androgenisation, show a more systematic cognitive style (Valla et al., 2010), whereas women with no androgenisation show 'E type' cognitive style or greater empathy (Wakabayashi et al., 2006). There is empirical data showing a high susceptibility for developing depression and neuroticism depending on a certain cognitive style, which means men with 'E type' style and women with 'S type'. A physiological indicator of this effect could be higher cortisol levels than people with a standard cognitive style (Nakayama, Takahashi, Wakabayashi, Oono & Radford, 2007).

The 2D:4D ratio has been related with psychological trait-variables such as aggressivity. In this respect, low ratio-values have been associated with physical aggression in men (Bailey & Hurd, 2005) and with reactive aggression in

women (Benderlioglu & Nelson, 2004). There is a greater level of cooperation in young adults of both genders with lower 2D:4D ratios (Millet & Dewitte, 2006). This indicator has been related to either the organising effects or activating effects of testosterone and cortisol. At a prenatal level, a predominance of the levels of testosterone compared to oestrogens in the amniotic fluid has been associated with the development of a smaller 2D:4D ratio (Lutchmaya, Baron-Cohen, Raggatt, Knickmeyer & Manning, 2004). The results on the relationship between testosterone and the 2D:4D ratio in adults are inconsistent, while in infertile men a negative correlation between both parameters has been described (Manning et al., 2004), whereas there is no relationship with the non-clinical population in both genders (Hönekopp, Barthold, Beier & Liebert, 2007). However, a recent study has shown that women (non-clinical population) with lower values of the 2D:4D ratio were susceptible to the effects of the exogenous administration of testosterone and decreased their scores for empathy (van-Honk et al, 2011). Moreover, there is also evidence that the 2D:4D ratio is related to other hormones such as cortisol. The levels of this hormone predicted values of the 2D:4D ratio for the right-hand in non-clinical populations of both genders (Beaton, Rudling, Kissling, Taurines & Thome, 2011) although the significance of this relationship has not yet been clarified.

These indicators of androgenisation have been used in general for the analysis of clinical populations such as people with ASD, but a possible use to characterise the idiosyncrasies of their parents has been neglected. It has been hypothesised that these people have, to a greater extent than the general population, autistic traits reflected in lower values of the 2D:4D ratio and high scores on the autism quotient (Wheelwright, Auyeung, Allison & Baron-Cohen, 2010). This data allows us to frame this population in the broad autism phenotype (BAP) (Manning, Baron-Cohen, Wheelwright & Sanders, 2001) and they are supported by a genetic predisposition in the etiology of these disorders (Buxbaum, Baron-Cohen & Devlin, 2010).

Considering the evidence that could refer to an idiosyncratic endophenotype of parents of offspring with ASD, the purpose of this paper is to examine if the parents of offspring with ASD have idiosyncratic features that differentiate them from the general population, using different markers of androgenisation, and with a particular emphasis on the 2D:4D ratio. Another aim is to analyse whether this indicator could be used as a predictor of other psychobiological variables related with androgenisation and the severity of ASD. It is hypothesized that parents of people with ASD would be more androgenised than the normal population. Accordingly, they would be framed in the BAP as the 2D:4D ratio is a good indicator of the condition (Manning et al., 2001). Furthermore, in line with the results obtained in previous studies at a cognitive level (Baron-Cohen, 2010b) we hypothesise that this indicator does not differ when comparing parents of people with ASD, given its high androgenisation. Finally, the 2D:4D ratio predicts the severity

of the symptoms of offspring with ASD, so a lower ratio would be associated with more severe symptoms (Manning & Bundred, 2000).

Methods

Participants

The final sample consists of 85 people aged between 31 and 63 years *(M = 44.27, SD = 6.58)* and with a body mass index (BMI) of *M = 26.80, SD = 4.70* kg / m², who participated voluntarily and signed an informed consent in accordance with ethical standards for human research. The experimental group consisted of 43 subjects, all fathers *(n = 16)* or mothers *(n = 27)* of offspring with ASD. The control group consisted of 42 fathers *(n = 19)* and mothers *(n = 23)* with similar characteristics to the experimental subjects, and whose children do not suffer any ASD or other chronic disorders. The ages of the offspring with ASD ranged from 5 to 30 years *(M = 14.25, SD = 5.73)*, with a gender distribution of 34 men and 7 women.

Procedure

Participants were scheduled to attend the laboratories of the psychology faculty at the University of Valencia, having previously been asked not to eat food or drink (except water, brushing teeth, or chewing gum) during the two hour period before arrival at the laboratory. The experimental sessions lasted approximately an hour and a half and were carried out between 16:00 and 19:00 pm (when cortisol levels are most stable) (Dickmeis, 2009).

After arrival in the laboratory, subjects were taken to a room with a constant temperature (22±1°C) and the anthropometric variables including weight, height, 2D:4D ratio, and socio-demographic variables were recorded. Two samples of saliva were then collected for determining hormone cortisol (Csal) and testosterone (Tsal), the first of the sample was taken at the end of the collection of anthropometric data and the last collection was within 20 minutes. Participants then completed questionnaires that assessed the psychological dimension feature type and, in the case of parents of offspring with ASD, a questionnaire aimed at assessing the degree to which their children suffer from autism.

Variables and measurement instruments

2D:4D ratio

To calculate the 2D:4D ratio three separate measurements of the length of the index finger (2D) and the ring finger(4D) of both hands were made. Measurements were made on the ventral side of the hand. The length of the finger was taken from the proximal fold at the base of the finger to the tip thereof, as this measurement procedure has a high replication (Schneider et al., 2006). For this purpose we

used digital calipers with an accuracy of 0.01 mm. Two of the three measurements were made directly by two investigators, while the third was performed using a scanner for subsequent measurement. The value of the 2D:4D ratio for each hand was obtained by calculating the arithmetic mean of three measurements, as has been done in previous studies (Schneider et al., 2006). Furthermore, as an additional index of androgenisation, we obtained the directional asymmetry ratios of both hands (D_{r-l}). For this, the value of the 2D:4D ratio of the left hand to the right hand was subtracted (Rahman & Wilson, 2003).

Trait psychological variables

The *empathy quotient* (EQ) consists of 60 items that are distributed on a Likert scale from 0 to 2, 40 of which relate to empathy while the remaining 20 control-items did not count for obtaining the total score. The higher the score, the greater the empathy (Baron-Cohen & Wheelwright, 2004).

The *systematisation quotient* (SQ-R) consists of 75 items distributed so that 55 relate to the systematic and 20 are control items. Correction and interpretation of this survey is similar to those of EQ (Wheelwright et al., 2006).

Cognitive styles were calculated according to the formulas: E2 = (SQ-R-55,6) / 150; F2 = (EQ-44,3) / 80; G2 = (E2-F2) / 2. According to the value, G2 is classified as 'Extreme S' (G2 = .21), 'S Type' (G2 = .04), 'B Type' (G2 = 0); 'E Type' (G2 = - .021) and 'Extreme E' (G2 = - .21) (Wakabayashi et al., 2006).

The *adult autism questionnaire (AQ adults)* is composed of 50 items that quantify autistic traits. This self-administered questionnaire is often used to detect autistic features in adults such as Asperger syndrome or individuals with high functioning autism (Baron-Cohen, Wheelwright, Skinner, Martin & Clubley, 2001).

The version of the *autism questionnaire for adolescents (adolescent AQ)* consists of 50 items that quantify autistic traits. The higher the score the greater the severity of the symptoms. This is an adapted version of the autism spectrum coefficient for children and adolescents aged 9 to 16, although it is also valid for older people (Baron-Cohen, Hoekstra, Knickmeyer & Wheelwright, 2006) and is designed to be completed by parents or carers.

Empathy was assessed from the Spanish version of 'interpersonal reactivity index' (IRI) (Mestre, Frías & Samper, 2004) which consists of four subscales (perspective taking, empathic concern, fantasy, and personal distress) and is composed of 28 items which are scored on a Likert scale from 1 to 5.

Cooperativity was assessed with the subscale of cooperation of the revised Spanish version (Gutiérrez-Zotes et al., 2004), 'temperament and character inventory' (Cloninger, Svrakic & Przybeck, 1993). It consists of 37 items rated on a Likert scale of 1 to 5 grouped into six subscales: social tolerance, empathy, altruism, compassion integrity, and fellowship, and a final score obtained from the sum of the above.

Anger was assessed using the Spanish adaptation of 'anger expression inventory state-trait STAXI-II' (Miguel-Tobal, Casado, Cano-Vindel & Spielberger, 2001). We assessed either trait anger or anger expression. The questionnaire consists of 49 items distributed into six scales: two for trait anger (temperament and angry reaction), and four for the expression of anger (expression-in, expression-out, control-in and control-out). A general index of anger expression (IAE) being extracted from the last four scales.

Hormonal analyzes (Csal and Tsal)

The basal levels of hormones were obtained from two samples of saliva. The Csal was collected using a Salivette (Sarstedt, Rommersdolf, Germany) and Tsal directly by salivation through a glass tube. The samples were frozen at -20°C until analysis by radioimmunoassay and LIA, respectively.

The reactives used for Csal were count-a-count Cortisol (DPC, Siemens Medical Solutions Diagnostics). The samples were measured in duplicate and all those belonging to the same participant were included in the same assay. The coefficient of variation inter-duplicate maximum considered for the repetition of the determination was set at 8%. Assay sensitivity was 0.5 ng/dl. The coefficients of intra-and inter-assay variation were 2.8 and 5.3% respectively. All values are expressed in nmol / l.

The reactives used for the Tsal were the 'saliva testosterone ELISA Kit' testosterone (dbc-Diagnostics Biochem Canada Inc.). Chemiluminescence inmunoassay (LIA) was used for its determination. Assay sensitivity was 1 pg / ml. The coefficients of variation intra-and inter-assay were 3.98 and 7.98% respectively. All values are shown in pmol / l. Due to an insufficient volume of saliva from seven subjects, 37 experimental subjects (14 males and 23 females) and 41 control subjects (19 males and 22 females) were used in the data.

Data Analysis

Univariate ANOVAs were performed with between-subject factors 'group' and 'gender' for the 2D:4D ratio and the anthropometric variables (age and BMI), psychological (aggression, empathy and cooperativeness), and hormonal (Tsal and Csal). For the analysis of the frequencies of the sociodemographic and cognitive styles, chi-square statistics were used – it having been previously found that they are normally distributed, using the Kolmogorov-Smirnov statistic ($p <.001$).

The *a posteriori-tests* were performed using the Bonferroni test. Linear regressions were used to study the association between the 2D:4D ratio and androgenisation variables and the severity of symptoms of the children.

All statistical analyses were performed using SPSS 17.0 software, considering as significant less than .05 ($p < .05$). Less than .08 (p < .08) has been considered a trend to sig-

nificance. The values of the descriptive are expressed as mean and standard deviation (*M, SD*, respectively).

Results

Sample characterisation

Although there were no differences between groups in BMI, there was a significant effect of 'group', and the interaction 'group x gender' for age [F (1, 84) = 9.73, p <.05, F (1, 84) = 4.30, p <.05, respectively], so men in the control group were younger than other subjects (all p <.05). Socio-demographic groups differed in educational levels [X^2 (3, N = 85) = 9.56, p <.05], where most of the control subjects had university studies while the experimental group had secondary studies. However, the groups did not differ in the phase of the menstrual cycle (for women), medication, use of cigarettes, marital status, source of income, and total number of children living in the family home. Therefore, both age and educational levels were included as covariates in subsequent analyses. The values *(M, SD)* for anthropometric and sociodemographic variables as a function of group and gender of the participants are summarised in Table 1.

Table 1. Anthropometric and socio-demographic variables in terms of group interaction (parents of ASD or controls) and gender (men or women).

		ASD		CG	
		Men (*n*=16)	Women (*n*=27)	Men (*n*=19)	Women (*n*=23)
Age (years), *p≤0.05		46.31±5.80	46.15±7.66	39.32±4.70	44.74±5.01
BMI (kg/m²)		28.79±4.77	26.44±5.13	27.96±2.72	25.06±4.92
Left 2D:4D ratio		0.97±0.04	0.97±0.03	0.97±0.04	0.97±0.03
Right 2D:4D ratio		0.97±0.04	0.98±0.03	0.98±0.03	0.98±0.03
D_{r-l}		0.002±0.02	0.008±0.03	0.007±0.02	0.007±0.02
Phases of the menstrual cycle	LUTEAL(1-14)		12 (44.4%)		6 (26.1%)
	FOLLICULAR(15-menstrual period)		8 (29.6%)		11 (47.8%)
	AMENORRHEA (>6months)		7 (26%)		6 (26.1%)
Marital status	SINGLE	-	1 (3.7%)	1 (5.3%)	1 (4.3%)
	MARRIED	16 (100%)	24 (88.9%)	18 (94.7%)	20 (87%)
	DIVORCED	-	2 (7.4%)	-	-
	WIDOWED	-	-	-	2 (8.7%)
	OTHERS	-	-	-	-
Educational level	NONE	-	-	-	-
	BÁSICS	3 (17.6%)	7 (25.9%)	-	2 (8.7%)
	ADVANCED	6 (35.3%)	4 (14.8%)	-	5 (21.7%)
	UNIVERSITY	7 (41.2%)	15 (55.6%)	18 (94.7%)	16 (69.6%)
	OTHERS	-	1 (3.7%)	1 (5.3%)	-
Source of income	PENSION	-	1(3.7%)	-	1 (4.3%)
	JOB	13 (76.5%)	22 (81.5%)	18 (94.7%)	17 (73.9%)
	UNEMPLOYMENT P.	1 (5.9%)	-	-	2 (8.7%)
	OTHERS	2 (11.8%)	4 (14.8%)	1 (5.3%)	3 (13%)
Number of children	1	4 (23.5%)	6 (22.2%)	9 (47.4%)	5 (21.7%)
	2	11 (64.7%)	16 (59.3%)	8 (42.1%)	13 (56.5%)
	3	1 (5.9%)	3 (11.7%)	2 (10.5%)	4 (17.4%)
	4	-	1 (3.7%)	-	-
	5	-	-	-	1 (4.3%)
	6 or more	-	1 (3.7%)	-	-

Differences between parents with and without children diagnosed with ASD

There were no significant effects for the 2D:4D ratio and D_{rl} (Table 1). The empathy quotient (EQ) had a significant effect of factor 'group' [F (1, 84) = 3.87, p <.05], so that parents of people with ASD had lower EQ than the control subjects. For the systematisation quotient (SQ-R) there was a significant effect of 'gender' [F (1, 84) = 14.16, p <.001] showing a higher ratio of men than women. These results were also found in the case of adult autism quotient (AQ adults) [F (1, 84) = 4.24, p <.05] (Table 2).

Although the groups differed only in the ratio of empathy, we observed a differential distribution of the same, since in the experimental group subjects were similarly distributed between cognitive styles E (34.88%), B (41.86 %) and S (23.25%). However, in the control group most of the subjects were concentrated in the type B (61.90%) (Table 2). Moreover, the gender factor was shown to have a significant effect on the distribution of the styles [X^2 (2, N = 85) = 8.07, p <.05], the main differences being in the types S and E, predominantly among men (37.14%) and type E among women (30%).

Table 2. Distribution of cognitive styles and psychological trait-variables in terms of group and gender.

		ASD		CG	
		Men (*n*=16)	Women (*n*=27)	Men (*n*=19)	Women (*n*=23)
Cognitive style	Extreme S	-	-	-	-
	S type	7 (43.75%)	8 (29.63%)	6 (31.57%)	3 (13.04%)
	B type	8 (50%)	10 (37.03%)	12 (63.15%)	14 (60.87%)
	E type	1 (6.25%)	9 (33.33%)	1 (5.30%)	6 (26.09%)
	ExtremeE	-	-	-	-
EQ, *p≤.05		36.69±10.37	42.70±11.33	44.53±7.36	45.26±7.18
SQ-R, *p≤.05		67.69±21.35	49.37±19.75	65.05±21.04	54.78±15.30
AQ adults, *p≤.05		18.88±6.30	15.41±6.29	14.58±4.93	13.69±4.02
IRI	Perspective taking	24.93±4.17	25.11±4.96	24.11±3.38	25.91±4.10
*p≤.05	Personal distress	16.56±5.42	17.19±4.78	14.11±2.71	15.78±3.98
(t)p≤.08	Empathic concern	26.93±3.79	28.07±3.99	23.32±4.11	28.00±4.49
	Fantasy	21.81±5.99	20.89±6.95	18.37±3.70	21.39±5.24
STAXI-2	External expression*	11.12±3.18	11.37±3.41	9.68±2.21	10.09±3.32
*p≤.05	Internal expression	12.94±4.48	11.74±3.17	11.26±2.66	11.13±4.26
	External control*	17.63±4.68	15.63±4.99	16.68±3.38	17.49±3.88
	Internal control*	12.31±4.63	12.25±3.80	15.26±4.44	16.08±4.18
	IEI*	30.13±10.13	31.22±10.02	22.00±8.41	23.69±9.84
	Temperament	2.38±2.68	2.96±3.06	1.89±2.05	1.83±1.85
	Reaction	7.00±3.77	6.70±2.93	5.95±2.91	6.43±3.26
TCI-R	Friendship*	27.06±4.63	27.93±3.74	25.11±4.94	31.22±2.35
*p≤.05	Altruism*	30.94±3.56	31.48±3.66	29.11±3.98	32.08±3.23
	Integrity*	29.88±4.99	31.56±3.23	28.84±4.51	31.78±4.81
	Empathy*	17.13±2.58	18.29±2.49	18.16±2.00	19.13±2.36
	Tolerance	30.50±4.19	31.74±3.57	30.74±3.16	32.39±3.70
	Total	27.10±3.17	28.20±2.08	26.39±2.31	29.32±1.91

With regard to empathy, there was a trend towards significance for interaction 'gender x group' on the fantasy scale [F (1, 84) = 3.31, p <.07] so that the male ASD group scored higher than the rest of the subjects. On the empathic concern scale either 'group' factor or 'group x gender' interaction showed a trend to statistical significance while the 'gender' factor had a statistically significant effect [F(1, 84) = 3.11, p < .08, F(1, 84) = 3.41, p < .07, F(1, 84) = 9.72, p < .005, respectively]. Parents of people with ASD showed more empathic concern than controls, and women generally scored higher on this scale than men. In addition, men in the control group received the lowest scores, the differences being statistically significant compared to other subjects (all p <.05).

With regard to cooperation, there was a significant effect of the interaction 'group × gender' for the fellowship scale [F (1, 84) = 8.14, p <.05], so that women in the control group had higher scores than the other groups (p <.05). The factor 'gender' was significant for all subscales except for tolerance and the total questionnaire score [F (1, 84) = 14.50, p <.001, F (1, 84) = 3.75, p <.05, F (1, 84) = 5.45, p <.05, F (1, 84) = 4.81, p <.05, and F (1, 84) = 13.90, p <.001] for altruism, integrity, empathy and fellowship – with higher scores in women.

Although there were no significant effects for trait aggression, the factor 'group' was statistically significant in the case of external expression, external control, internal control, and the rate of expression of anger [F (1, 84) = 6.07 , p

<.05, F (1, 84) = 4.41, p <.05, F (1, 84) = 11.79, p <.001, and F (1, 84) = 11.11, p <.001, respectively]. Parents of people with ASD had higher scores for expression-out and the rate of expression of anger; while control subjects scored higher on the control-out and the control-in of anger. In addition, there was a significant effect of 'gender' for external control of anger [F (1, 84) = 5.13, p <.05] and men had higher scores than women (Table 2).

Although there were no differences between groups, the factor 'gender' showed a significant effect for Tsal and Csal [F (1, 77) = 56.50, p <.001, F (1, 84) = 4.27, p <.05] with levels higher in both cases in men. The average values for both hormones according to the group and gender are presented in Table 3.

Table 3. Hormonal levels of Tsal and Csal in terms of group and gender.

	ASD		CG	
	Men (*n*=16)	Women (*n*=27)	Men (*n*=19)	Women (*n*=23)
Tsal (pmol/l)	64.45±28.22 (*n*=14)	26.17±20.29 (*n*=23)	59.29±29.50 (*n*=19)	25.36±35.1 7 (*n*=22)
Csal (nmol/l)	2.06±1,95 (*n*=16)	1.59±0.91 (*n*=27)	2.47±2.20 (*n*=19)	1.55±1.06 (*n*=23)

Patterns of prediction of the D2:D4 ratio in terms of being a parent or not of offspring with ASD

In the case of parents of offspring with ASD, the 2D:4D ratio of the left hand explained 12.4% and 10.3% of the total

variability of the scores of the empathy questionnaire (EQ) and autism (AQ adults) [β = 0.352, p <.05, β = - .321, p <.05, respectively]. It also explained 11.8% and 12.9% of the level of integrity and TCI total score [β = .344, p <.05, β = .359, p <.05], respectively, and the 10.6% of basal Csal [β = - .325, p <.05]. This pattern of relationship was not significant in the control group. The ratio D2:D4 of the right hand explained 12.9% and 9.3% of the expression-out and the control-out of anger [β = - .360, p <.05 and β = .305, p <.05, respectively and 10.2% of the level of integrity of TCI [β = .319, p <.05]. By using the index as a predictor D_{r-l} asymmetry was not explained by any of the variables of androgenisation.

In controls, the left D2: D4 ratio predicted 9.2% of the IRI fantasy scale [β = .303, p <.05] while the right explained 12% and 13% of the scores on the perspective-taking and fantasy subscales in this questionnaire [β = .346, p <.05, β = .361, p <.05, respectively]. The asymmetry index D_{r-l} explained 9.2% of the cognitive styles [β = 0.303, p <.05] and 12% of the expression-out of anger [β = .346, p <.05].

The D2:D4 ratio as a predictor of the severity of ASD

As shown in Figure 1, the 2D:4D ratio of the left hand in men explained 32.6% of the variability of the values of AQ for their children [β = - .571, p <.05].
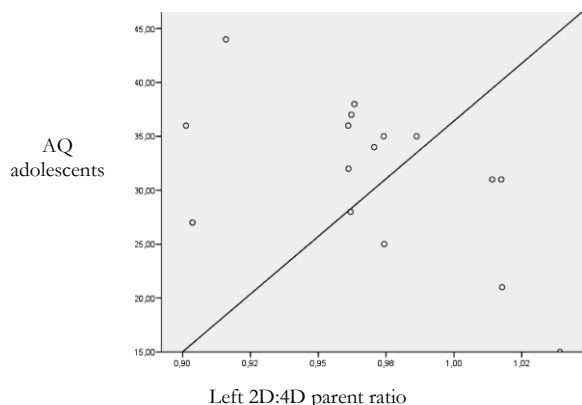

Figure 1. 2D:4D as predictor of the severity of the symptoms of their children

Discussion

Parents of offspring with ASD show similar values for the D2:D4 ratio, cognitive styles, and basal hormone levels as the control group. However, they are less empathic and have less control over the expression of anger. The D2:D4 ratio of this population predicts cognitive variables (EQ and AQ), behavioural (cooperativeness and expression of anger) and hormonal (Csal) of androgenisation. It is also a sensitive predictor of the severity of ASD for their children.

The values of the 2D:4D ratio for parents of individuals with ASD are similar to the control group, unlike the finding

in a previous study conducted in this population (Manning et al., 2001). In general, this index is 0.25 *SD* higher in women than in men (Manning et al., 2000), but our study does not reveal these differences (as we had hypothesised). Although the 2D:4D ratio cannot be considered as a deterministic parameter of prenatal androgenisation, it is a useful indicator when analysing its relationship with other variables. In this regard, it has proven useful in explaining human behaviour and cognition (van-den-Bergh & Dewitte, 2006). This study has shown that the 2D:4D ratio of parents of ASD predicts a greater number of variables related to the androgenisation than the control group. It has been suggested that the right-hand 2D:4D ratio is more sensitive to the effects of prenatal testosterone, and so it has a greater ability to predict cognitive variables, while behavioural and physiological variables are somewhat androgenised (Hönekopp & Watson, 2010). The 2D:4D ratio predicts more left than the right indicators on parents of offspring with ASD, and that includes empathy, the ratio of autism, cooperation and cortisol. No conclusive results affirm that the left-hand 2D:4D ratio is not sensitive to the effects of prenatal androgens. Thus, our results reinforce those found in other studies that show relationships or predictions associated with the left indicator (Bull & Benson, 2006).

The ratio of both hands was useful in predicting cooperation variables, although the results are not consistent with those described in a previous study (Millet & Dewitte, 2006), since in our study a higher 2D:4D ratio is related in cooperative strategies. These differences may be partly due to the type of measurement used to evaluate cooperation, because in the work mentioned previously the social dilemma game *Repeated public good game* was used, whereas in our work a questionnaire evaluation was used. In addition, while the left-hand 2D:4D ratio predicted empathy and autism spectrum quotient in adults, the right predicted anger expression. A higher 2D:4D ratio meant more empathy and less autistic traits. Although the ratio does not predict trait anger, a lower value for this indicator is associated with the expression-out and the control-out of anger. These results reinforce those described in previous studies which associated a lower value of the 2D:4D ratio with increased physical aggression in men (Bailey & Hurd, 2005) and reactive aggression in women (Benderlioglu & Nelson, 2004).

It is proposed that the 2D:4D ratio could be used as an indirect indicator of a predisposition to certain diseases such as autism, heart disease, or certain types of cancer (Manning & Bundred, 2000). But the fact that the 2D:4D ratio for the male parent is a predictor of the severity of the symptoms of children suggests that greater prenatal exposure to androgens in men may make them more susceptible to developing autism and increase the likelihood that their offspring will develop ASD. In this way, the 2D:4D ratio is characterised by high heritability through the male line, which increases the possibility that genes related to the Y chromosome may influence its expression (Voracek & Dressler, 2009). How-

ever, further studies would be necessary to employ more variable androgenisation to replicate these results.

Cognitive styles in the entire sample was divided into types S, B, and E; whereas there are no subjects in the extreme types. However, the percentage of S-type is slightly higher in ASD parents than in controls. These results are consistent with expectations because they are not individuals with ASD but first-degree relatives. Although the parents of persons with ASD show a systematic quotient similar to the controls, their empathy quotient is lower. A previous study conducted in this population showed less activation of the amygdala and the fusiform gyrus in a test of empathy consisting of recognition of emotional expression in the eyes (Greimel et al., 2010). However, the analysis of empathy through the IRI subscales in our data indicates that there is more imagination and empathic concern in parents of people with ASD than in control subjects. Although a priori these findings may seem contradictory, it should be noted that empathy is a multidimensional construct (Derntl et al., 2010), so the differences may be specific and refer to specific aspects.

Although there is no scientific proof that parents of people with ASD have higher levels of aggression, our results indicate that they do not show more aggressive traits – but do show less control and greater expression of anger. According to the above, the same people may behave aggressively in some situations; but also express empathic behaviour. As a current theory holds, very empathetic people can also be very aggressive and vice versa, although they cannot manifest both behaviours at the same time, due in part to activating similar neuroanatomical structures (Moya-Albiol, Herrero & Bernal, 2010). It is therefore fitting that people who have trouble controlling their anger, can also express concern for others.

Androgenisation has often been associated with socially undesirable behaviour such as violence, but it has also been claimed that a strategy to gain status characterised by high androgenisation is cooperation (Millet & Dewitte, 2006). Cooperation as it has been evaluated in our study did not differentiate between groups, but has been revealed that women, primarily those in the control group, showed higher scores.

When compared by gender, men were more systematic, show more autistic traits, and were less empathetic and cooperative, as described in previous studies (Moya-Albiol et al., 2010). Our data does not reveal differences between parents of offspring with ASD and control hormone levels, although as expected there are gender differences for Tsal.

There are also differences for Csal, and this result could be explained by differences in age, menstrual cycle phase in women, or that some subjects are postmenopausal (Kudielka, Buske-Kirschbaum, Hellhammer & Kirschbaum, 2004). Another possibility is a differential pattern of cortisol for each gender linked to the situation of caring for people with ASD, something that would be worth examining in future studies.

The main limitation of this study is that it is cross-sectional, so that individual differences may overlap other effects to be evaluated in a single moment in time in the life of such persons. However, given that the personal situation of the participants it is extremely difficult to conduct longitudinal studies.

In order to characterise the idiosyncrasies of the sample it would be interesting to extend the number of variables taken into account. This would mean making an analysis from a holistic perspective, in line with the work we have been doing in our laboratory (De Andres-Garcia et al., 2011, Gonzalez-Bono, De Andres Garcia & Moya-Albiol, 2011, Serrano, Moya-Albiol & Salvador, 2009). It would also be useful to analyse other samples of parents in which prenatal androgenisation had neuroprotective effects when developing the disorder in children, as in the case of eating disorders (Klump et al., 2006). Thus, the 2D:4D ratio is a good indicator to characterise the idiosyncrasies of the parents of people with varying degrees of androgenisation.

In conclusion, this study has shown that the 2D:4D ratio and asymmetry by themselves are not significant predictors of a genetic trait of autism in the clinical setting. However, they have considerable predictive value in explaining the behavioural and cognitive variables that characterise the parents of offspring with ASD, and serve as a bridge between idiosyncrasy and severity of ASD children. However, given the relatively low percentage of variance explained, an integrated approach should be used in which the 2D:4D ratio is another element in a continuous interaction with the various elements that describe the androgenisation. Furthermore, the subjects are not individuals with ASD, but direct ancestors – which mean that the effects and differences in androgenisation parameters described throughout the study are subtle and require further analysis.

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