

Spatial abilities are not related to testosterone levels and variation in the androgen receptor in healthy young men

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Abstract. Androgens modulate brain functions such as cognition, emotions and ability. Several studies have shown a correlation between testosterone levels and mental rotation. The aim of the present study was to confirm the influence of salivary testosterone levels, 2D/4D ratio (such as a putative marker of prenatal testosterone), and sensitivity of androgen receptor on the mental rotation in healthy young men. Seventy-five healthy young men (age, 21.86 year) volunteered in this study. Mental rotation scores of our subjects were assessed using the Vandenberg and Kuse Mental Rotation Test. The 2D/4D finger length ratio as an indicator of prenatal testosterone was used as an average measurement of both hands. Correlation analysis revealed no correlation between salivary testosterone levels and mental rotation. However, we have observed a trend towards a negative correlation. There were no statistically significant results between 2D/4D ratio and mental rotation or between polymorphic three-nucleotide (CAG) repeats and mental rotation tests. Future studies should focus on other genetic determinants of spatial abilities, potentially genes involved in testosterone metabolism.

Key words: Testosterone — Mental rotation — Androgen receptor — 2D/4D ratio

Introduction

There is compelling evidence that early androgen exposure is important for brain development and sexual differentiation in humans (Knickmeyer et al. 2011). There are two periods in early life during which the male brain is exposed to extremely high levels of androgens, during midgestation and during first few months of postnatal life (Beaton et al. 2011). Androgens modulate brain functions, such as cognition, emotions and behavior, that show sex differences, including sexual orientation, core gender identity, and some sex-related cognitive and personality characteristics (Hines et al. 2011).

Mental rotation (MRT) is the ability to imagine objects from a perspective other than the one depicted. Several studies have found a significant positive relationship between ac-

tually measured testosterone levels and spatial ability scores (Silverman et al. 1999; Moffat et al. 2002; Hooven et al. 2004; Driscoll et al. 2005). Effects of testosterone supplementation on spatial memory have been also reported in healthy men (Cherrier et al. 2001). However, there are some studies that found no significant relationship between spatial skills and sex hormones (Bhasin et al. 2001; Fonda et al. 2005; Halari et al. 2005; Martin et al. 2008; Matousek and Sherwin 2010). Negative correlations between spatial skills and testosterone levels have been found by others (Ostatnikova et al. 2002; Yonker et al. 2006; Martin et al. 2007). Relationship between testosterone and spatial ability has also been studied in young adults. Puts et al. (2010) concluded that there is no consistent evidence that testosterone has activational effects on spatial ability. They did not find any significant association between testosterone levels and mental rotation in a large sample of young adults with an average age 22. It was suggested that circulating testosterone does not predict mental rotation in young men, but its influence may be earlier, during the prenatal period (Puts et al. 2010). Organizational effect was supported by Heil et al. (2011) and Vuoksimaa et al. (2010).

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They studied females from the opposite-sex (OS) and twin pairs *vs.* females from the same-sex (SS). Data were fully consistent with the hypothesis that increased prenatal testosterone exposure in females masculinizes mental rotation performance with OS female twins having a higher mental rotation performance than SS female twins (Vuoksimaa et al. 2010; Heil et al. 2011). The organizational effect of testosterone is also supported by studies in patients with congenital adrenal hyperplasia (CAH). Patients with CAH are consequently masculinized. Some studies have found CAH females to exhibit masculinized spatial abilities (Hines et al. 2003).

Androgens and estrogens differentially regulate the genes that control chondrocyte proliferation, leading to differential growth of the fourth digit in males and females (Zheng and Cohn 2011). Manning et al. (1998) introduced the length ratio of the second (index) finger to the fourth (ring) finger known as the 2D/4D ratio (Manning et al. 1998). High concentrations of fetal testosterone lead to a lower 2D/4D ratio, based on which it is assumed that there is increased prenatal testicular activity. The typical outcome is that high scores on a mental rotation test are associated with low values of the 2D/4D ratio for males. The relationship between 2D/4D and spatial ability has not been robustly demonstrated in previous studies. Significant associations have been reported between mental rotation accuracy and 2D/4D, particularly in men (Manning and Taylor 2001; McFadden and Shubel 2003). The effect of androgens is dependent not only on their current levels, but also on their metabolism. At the molecular level, the effect of androgens is mediated through the activation of androgen receptor (AR). A polymorphic three-nucleotide (CAG) repeats in exon 1 encodes a polyglutamic tract. The normal number of repeats is between 11 and 35 (Greenland and Zajac 2004). A reduced number is associated with higher AR transcriptional activity and, therefore, stronger androgen action (Tut et al. 1997). Several studies have shown that the number of CAG repeats directly correlates with serum testosterone levels, indicating that the weaker AR activity may indeed be compensated by higher androgen levels (Krithivas et al. 1999; Crabbe et al. 2007; Huhtaniemi et al. 2009).

The primary aim of our study was to confirm the influence of prenatal (such as 2D/4D ratio) and salivary testosterone levels and the sensitivity of androgen receptor on the mental rotation in healthy young men. We expected a negative correlation between salivary testosterone and mental rotation as found in our previous studies (Celec et al. 2002; Ostatnikova et al. 2010). As a secondary aim we investigated the influence of all measured parameters and their interaction on mental rotation performance. We also investigated the relationships between salivary testosterone levels and CAG repeats and 2D/4D ratio and the relationship between CAG repeats and 2D/4D ratio of both hands.

Materials and Methods

Participants

Seventy-five healthy young men age 21.86 ± 1.65 year (mean \pm SD) volunteered and provided written informed consent prior to their participation in this study. All volunteers were second year medical students at Comenius University in Bratislava. Exclusionary criteria were hormonal disorders in the subjects. All the participants were Caucasians and citizens of Slovakia. All procedures were approved by the Ethics Committee of the Comenius University Faculty of Medicine.

Sample collection

The probands were requested to collect whole saliva samples in a sterile tube (Sarstedt, Nümbrecht, Germany) between 8:00 and 10:00 am conforming to the circadian rhythm of testosterone. Volunteers were requested not to eat, drink or brush their teeth 30 minutes before the collection procedure.

Genetic analyses

Genomic DNA from saliva was extracted using the silica membrane based kit (Qiagen, Hilden, Germany) following the manufacturer's instructions (QIA amp DNA Blood Mini Kit Handbook 04/2010) according to DNA purification protocol for blood/body fluids.

Polymerase chain reaction (PCR) and electrophoresis were used to determine the number of microsatellite CAG repeats within Exon 1 of the Androgen Receptor gene (*AR CAGn*).

PCR was performed in 20 μ l volume with 250 nmol/l primers: forward: 5' GCGCGAAGTGATCCAGAAC 3' tagged with 6-carboxyfluorescein and reverse 5' CTCATC-CAGGACCAGGTAGC 3', 1 \times Taq buffer (Fermentas, Vilnius, Lithuania) and 1U of Taq DNA polymerase (Fermentas, Vilnius, Lithuania). The PCR program included: initial denaturation at 94°C for 4 min, followed by 35 cycles each consisting of denaturation at 94°C for 45 s, annealing at 59.5°C for 45 s and polymerization at 72°C for 45 s. The length of the final fragment was 181 bp. The number of repeats was analyzed by capillary electrophoresis.

Hormonal analyses

Testosterone levels were determined by ELISA assay using a commercial Salivary Testosterone ELISA kit according to the manufacturer's instructions (DRG Instruments GmbH, Marburg, Germany). The intraassay coefficient of variation was 4.3% and interassay 7.2%.

Measurement of 2D/4D ratio

Probands provided both hands to make a digital scan of the ventral surface. The lengths of second and fourth digit were measured and ratio was calculated using AutoMetric software in the left and right hand independently by two investigators and averaged.

Spatial abilities

An instrument widely used to assess the mental rotation test (MRT; Vandenberg and Kuse 1978), involves the comparison of 3D block figures. MRT was performed on all participants immediately after the collection of saliva in the morning between 8:00 to 10:00 am. This test measures one's capacity for mental manipulation and rotation of geometric shapes. Participants were provided with an image of a three-dimensional geometric object and asked to determine what the object would look like if it had been rotated in space choosing two correct responses from the four given possibilities for each item. If both marked figures were correct, participants were given one point, yielding a possible MRT score range from 0 to 20. The MRT was administered in two parts (I and II), each consisting of 10 trials. Coefficient K was calculated according to the formula: $K = \Psi^2 / (\rho \times \omega)$; where Ψ is the number of correct answers, ρ is the number of answered trials and ω is the total number of trials. The time limit for each part was 3 minutes with a break between the two parts. There was 5-min practice period including four trials before the actual test.

Statistical analyses

For statistical analysis GraphPad Prism 5 was used. Data are presented in the form of a mean \pm SD. Linear correlation and linear regression analyses were conducted. Correlations of prenatal (assessed by 2D/4D ratio) and salivary testosterone levels with biological parameters were tested using the parametric Pearson's correlation test. Results of simple correlation between all variables are described by a correlation coefficient (r). In form the results of linear regression showing the associations between biological parameters and mental rotation performance are described and shown as a coefficient of determination.

In addition, a multifactorial linear regression was used for the analysis of effects of the particular factors on MRT.

Results

Seventy-five healthy young men participated in this study, with average salivary testosterone levels of 0.599 ± 0.263 nmol/l (Table 1). Average coefficient mental rotation scores

Table 1. Descriptive statistics of observed parameters in healthy young men

Parameter	Value	
Age (years)	21.860 ± 1.650	
Testosterone level in saliva (nmol/l)	0.599 ± 0.263	
Coefficient of mental rotation	0.383 ± 0.208	
Raw score of mental rotation	correct answers	
	part I	4.884 ± 2.118
	part II	4.580 ± 2.291
	total correct answers	8.824 ± 4.500
incorrect answers	part I	1.261 ± 1.009
	part II	1.826 ± 1.350
	total incorrect answers	2.878 ± 1.951
2D/4D ratio	right hand	0.979 ± 0.035
	left hand	0.975 ± 0.033
	averaged ratio	0.959 ± 0.035
(CAG)n (number or repeats)	21.541 ± 3.123	

Value are mean \pm SD.

of our subjects were 0.383 ± 0.208 (Table 1). The average raw score of correct answers was 4.884 ± 2.118 in part I and 4.580 ± 2.291 in part II. The average raw score of incorrect answers was 1.261 ± 1.009 in part I and 1.826 ± 1.350 in part II (Table 1). The average raw score of total correct and incorrect answers was 8.824 ± 4.500 and 2.878 ± 1.951 respectively.

Salivary testosterone levels did not correlate significantly with mental rotation ($r = -0.210$, $p = 0.093$; Fig. 1A). However, we observed a trend toward negative correlation. We expected a negative correlation as in our previous studies (Celec et al. 2002; Ostatnikova et al. 2010). CAG repeats also did not correlate with mental rotation ($r = 0.059$, $p = 0.666$; Fig. 1B). These findings don't confirm the activational effect of testosterone, however, neither the organizational effect of testosterone. 2D/4D ratio as a measure of prenatal level of androgens did not correlate with mental rotation in both hands, for the left hand ($r = -0.133$, $p = 0.368$), and for the right hand ($r = -0.115$, $p = 0.441$; Fig. 1C and D).

Correlation analysis revealed no correlation between salivary testosterone levels and sensitivity of androgen receptor ($r = -0.044$, $p = 0.735$; Fig. 2A). We expected that higher numbers of CAG repeats (weaker AR activity) will be compensated by higher testosterone levels, but the study did not confirm it. We also did not find any correlation between current and prenatal levels of testosterone. Salivary testosterone levels did not correlate with 2D/4D ratio in both hands: for the left hand ($r = 0.018$, $p = 0.902$) and for the right hand ($r = 0.037$, $p = 0.806$; Fig. 2B and C). Sensitivity of androgen receptor (CAG repeats) did not correlate neither, with 2D/4D ratio in the left hand ($r = 0.011$,

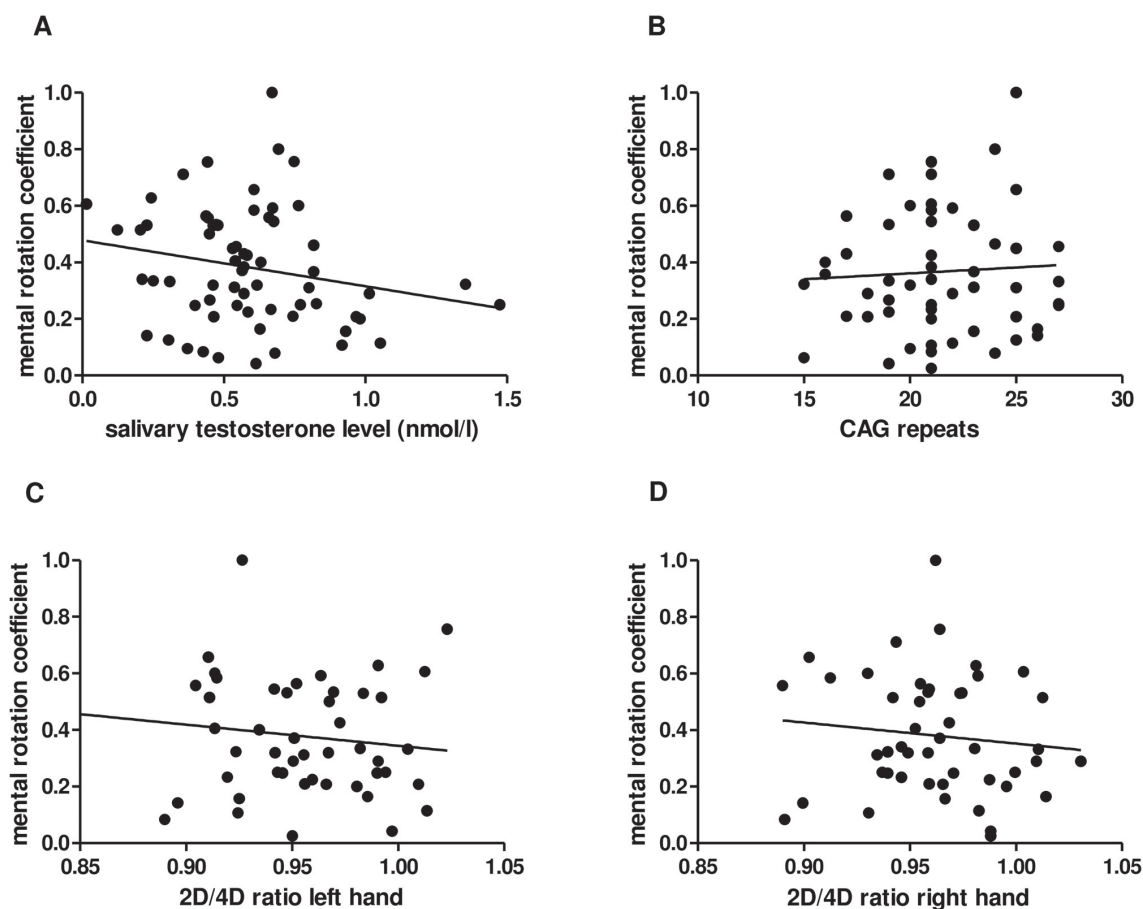


Figure 1. Correlation analysis between mental rotation coefficient and salivary testosterone levels and 2D/4D ratio and sensitivity of androgen receptor. **A.** Actual salivary testosterone levels did not correlate significantly with mental rotation ($r = -0.210$, $p = 0.093$). **B.** CAG repeats did not correlate with mental rotation ($r = 0.059$, $p = 0.666$). 2D/4D ratio such as retrospective correlate of androgens did not correlate with mental rotation in both hands, for the left hand ($r = -0.133$, $p = 0.368$; **C**), for the right hand ($r = -0.115$, $p = 0.441$; **D**).

$p = 0.945$; Fig. 2D) and the right hand ($r = 0.043$, $p = 0.794$; Fig. 2E) respectively.

We have done linear model analysis testing what impact all measured parameters and their interaction have on MRT. The effect of actual salivary testosterone on MRT in multifactorial analysis was not significant ($F = 3.496$, $p = 0.069$). Similarly, excluding 2D/4D from the interaction leads to no effect of salivary testosterone on MRT ($F = 1.844$, $p = 0.180$). Exclusion of CAG repeats from the analysis led to a significant association between testosterone and MRT ($F = 4.938$, $p = 0.031$).

Discussion

This study was undertaken to examine the relationship between testosterone and mental rotation in a sample of 75 healthy young men. Contrary to our hypothesis, statistical

analyses failed to reveal significant associations between salivary testosterone levels and performance of tasks for spatial abilities (Fig. 1A). In accordance with our study, Puts et al. (2010) also did not find a relationship between the salivary testosterone levels and mental rotation in young men (Puts et al. 2010). Based on these results we suspect that the effect of testosterone is earlier, during prenatal development. This organizational effect of testosterone is supported by Grimshaw et al. (1995), although their results are not entirely clear. Prenatal testosterone was measured from amniotic fluid between 14–20 weeks of pregnancy and children were tested at the age 7. A significant positive association between fetal testosterone levels and faster performance on a mental rotation task was found in a subgroup of girls whose response times correlated with the angle of rotation, indicating they had used a rotational strategy (Grimshaw et al. 1995). However, there is also a finding by Auyeung et al. (2012), observing no significant relationship

between mental rotation ability and fetal testosterone levels (Auyeung et al. 2012).

Opposite organizational effects of testosterone is an activational effect supported by studies that found relationship between actual levels of testosterone and spatial abilities (Thilers et al. 2006; Yonker et al. 2006; Martin et al. 2007; Hausmann et al. 2009). In the present study we expected that salivary testosterone levels will negatively correlate

with mental rotation as in our previous studies (Celec et al. 2002; Ostatnikova et al. 2010) or in studies in young men (Moffat and Hampson 1996; Vuoksima et al. 2012). We have seen a trend towards a negative correlation, but we have not confirmed a predicted negative correlation probably due to the small number of subjects. Another possible explanation could be that testosterone influences cognition *via* its metabolites estradiol or dihydrotestosterone (Celec

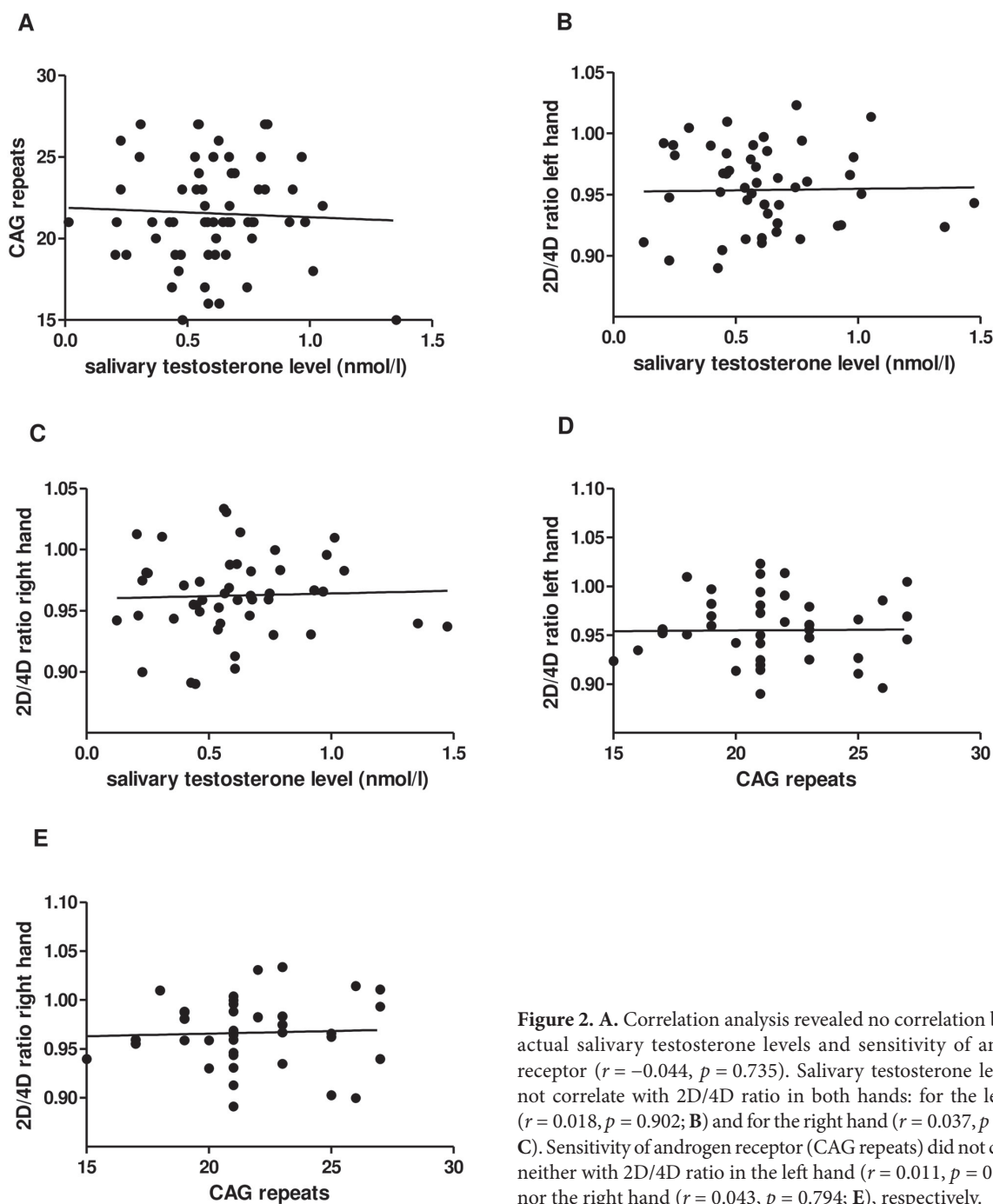


Figure 2. A. Correlation analysis revealed no correlation between actual salivary testosterone levels and sensitivity of androgen receptor ($r = -0.044$, $p = 0.735$). Salivary testosterone levels did not correlate with 2D/4D ratio in both hands: for the left hand ($r = 0.018$, $p = 0.902$; B) and for the right hand ($r = 0.037$, $p = 0.806$; C). Sensitivity of androgen receptor (CAG repeats) did not correlate neither with 2D/4D ratio in the left hand ($r = 0.011$, $p = 0.945$; D) nor the right hand ($r = 0.043$, $p = 0.794$; E), respectively.

et al. 2009; Kelemenova et al. 2010; Verhovshek et al. 2010). Ostatnikova et al. (2010) suggested that changes in spatial performance may depend on salivary testosterone fluctuations in both male and female subjects throughout a month, which would support the activational effects of currently circulating testosterone levels. Men achieved the best scores during the low-testosterone phase and women achieved the maximum score in the periovulatory phase (Celec et al. 2002; Ostatnikova et al. 2010). These findings support Courvoisier et al. (2013) in a longitudinal study, where the association between testosterone and performance differed across sexes (for males, it had an inverse U-shape, for females it was U-shaped) (Courvoisier et al. 2013). Abnormally high testosterone levels are linked with poor spatial ability. These results indicate that increasing testosterone levels do not necessarily lead to an amplification of male type characteristics. It seems that an optimal level of testosterone exists for certain cognitive domains (O'Connor et al. 2001).

The present study investigated the organizational effect of testosterone (through 2D/4D ratio) on mental rotation. The typical outcome is that high scores on a mental rotation test are associated with low values of the 2D/4D ratio for males. Significant associations have been reported between mental rotation accuracy and 2D/4D, particularly in men (Manning and Taylor 2001; McFadden and Shubel 2003). Current results agree with papers where correlation was not found (Fig. 1C and D). The relationship between 2D/4D and visual-spatial cognition has not been clearly demonstrated in previous studies, possibly due to the differences in methodology, small samples sizes, and different ages of subjects. In the future, large sample studies are necessary to reveal associations between 2D/4D ratio and spatial cognition that may bring significant and consistent results.

The effect of androgens is dependent not only on current levels, but also on their metabolism. Androgens act at the cellular level through the androgen receptor. Several studies documented a relationship between CAG polymorphism and cognitive abilities (Greenland et al. 2004). Lee and colleagues examined the association between cognition and AR CAG repeat length in older men. There was no correlation between CAG repeat length and fluid intelligence (Lee et al. 2010), which is consistent with our results (Fig. 1B). Our previous research indicated the association between functional polymorphism of the AR gene and intellectual giftedness in boys, but a number of CAG repeats was not correlated with any cognitive skills (Celec et al. 2013), however, authors revealed significant lower numbers of CAG repeats in gifted boys compared to controls. These results point towards a potential biological explanation of the relationship between testosterone and intelligence.

Previous studies focused on the relationship between testosterone levels and a number of CAG repeats. Several studies have shown that the number of CAG repeats directly

correlated with serum testosterone levels, indicating that the weaker AR activity (longer CAG segment) may indeed be compensated by higher testosterone levels (Crabbe et al. 2007; Huhtaniemi et al. 2009). We have not found correlation between CAG repeats and testosterone levels (Fig. 2A). Apparent discrepancies between studies may be underlined by differences in population genetic background, and the age of enrolled men. There is one study in young men, which did not find significant association between testosterone levels and a number of CAG repeats (Canale et al. 2005). These and our results suggest that the current testosterone levels are independent of androgen receptor sensitivity. It would be useful to investigate several other polymorphisms in the gene for androgen receptor, which could affect the sensitivity of AR. The major limitation of our study is a relatively small sample size.

The relationship between 2D/4D and fetal testosterone is clearly established. However, previous evidence of the relationship between the 2D/4D ratio and adult testosterone values is controversial. Only García-Cruz et al. (2012) found negative correlation between 2D/4D ratio with total, free, and bioavailable testosterone levels (García-Cruz et al. 2012). However, this study was undertaken involving men with an average age 67 years. Nevertheless, our results are consistent with the results of studies of healthy young men (Fig. 2B and C). Studies of healthy young men did not find any relationship between their hormonal profile and 2D/4D relationship. The mean age of males was between 18–25 years in the normal population (Bang et al. 2005; Hönekopp et al. 2007).

Some studies have reported the relationship between (CAG)_n in AR and 2D/4D ratio, Manning et al. (2003) first reported a correlation between the number of CAG repeats and 2D/4D ratio (Manning et al. 2003). Low CAG repeats and a low 2D/4D ratio indicate high activation of androgen-responsive genes. Marina et al. (2012) found positive correlation between CAG repeats and left hand digit ratio, with men with fewer CAG repeats having a significantly lower left hand 2D/4D ratio (Marina et al. 2012). We expected to find a positive correlation between 2D/4D ratio and number of CAG repeats in our subjects. However, we were not able to prove our hypothesis. Thus, we cannot confirm the relationship between prenatal testosterone levels and androgen receptor sensitivity (Fig. 2D and E). Our results are in accordance with other studies which have not found a significant relationship between polymorphism in AR and 2D/4D ratio (Hurd et al. 2010; Folland et al. 2012). Further studies employing more subjects are needed to clarify these inconsistent findings.

This study had two aims. The first aim was to investigate the effects of prenatal (*via* 2D/4D ratio) and salivary testosterone levels or sensitivity of androgen receptor on mental rotation in young healthy men. As we expected we

found that men with lower testosterone levels within the physiological range score better on tests of mental rotation, but these results were not statistically significant. Possible factors affecting our results were small sample size and also the fact that medical students are a group with highly specific interests, not representative of the general population and may affect their mental rotation ability. We assumed that mental rotation will also be affected by testosterone metabolism, but we were unable to demonstrate any effect. We have not found correlation between CAG repeats and testosterone levels. It is known that in the brain testosterone is converted to estradiol *via* aromatase. It is possible that decreased androgen sensitivity *via* a negative feedback mechanism indirectly affects estrogen receptor activation in some brain areas including those regions known to be important for spatial abilities. In the future it would be necessary to extend the analysis of genetic polymorphisms of the enzyme aromatase, which catalyzes the conversion of testosterone to estradiol.

A secondary objective of this study was to investigate the influence of all other variables and interaction between them on MRT performance. When we included all measured parameters the analysis, salivary testosterone levels had no effect on MRT performance. Similarly, the excluding 2D/4D from the interaction leads to no effect of salivary testosterone on MRT and the excluding a number of CAG repeats leads to significant effect of salivary testosterone on MRT. Taken together, it seems that testosterone partially determines MRT including its prenatal and actual salivary levels. Sensitivity of AR seems to influence salivary TST levels and thus may indirectly influence MRT performance.

In conclusion, our findings do not necessarily exclude the possible importance of the androgenic effect on the brain structure and function during the prenatal and postnatal period. These effects are shown in numerous studies showing that men score significantly better in tests of spatial skills than women. More studies that address the effects of androgens on specific cognitive abilities are needed to clarify this.

Conclusion

In healthy young men mental rotation is affected by neither the genetic polymorphisms of the androgen receptor nor by actual testosterone levels or 2D/4D ratio reflecting prenatal testosterone levels. Future studies should focus on other genetic determinants of spatial abilities, potentially in genes involved in testosterone metabolism.

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