Foetal testosterone and the child systemizing quotient

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Abstract

This study examines foetal testosterone (fT) levels (measured in amniotic fluid) as a candidate biological factor, influencing sex differences in systemizing. Systemizing is a cognitive process, defined as the drive to analyze or construct systems. A recent model of psychological sex differences suggests that this is a major dimension in which the sexes differ, with males being more drawn to systemize than females. Participants included 204 children (93 female), age 6–9 years, taking part in a long-term study on the effects of fT. The systemizing quotient – children’s version was administered to these mothers to answer on behalf of their child. Males (mean $Z = 27.79$, $G = 7.64$) scored significantly higher than females (mean $Z = 22.59$, $G = 7.53$), confirming that boys systemize to a greater extent than girls.

Stepwise regression analysis revealed that fT was the only significant predictor of systemizing preference when the sexes were examined together. Sex was not included in the final regression model, suggesting that fT levels play a greater role than the child’s sex in terms of differences in systemizing preference. This study suggests that the levels of fT are a biological factor influencing cognitive sex differences and lends support to the empathizing–systemizing theory of sex differences.

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Introduction

Male and female foetuses produce testosterone, with male foetuses producing more than 2.5 times the levels observed in females (1). In early prenatal life, testosterone enters the amniotic fluid via diffusion through the foetal skin, and later enters the fluid via foetal urination (2). Males are exposed to testosterone from the foetal adrenals and testes. The female foetus is also exposed to androgens but at lower levels. A small proportion may come from the foetal adrenals (a by-product of the production of corticosteroids) and some from the maternal adrenals, ovaries and fat (3).

In animal models, the critical period for sexual differentiation of the brain occurs when the differences in serum testosterone between sexes are highest (4). Studies reveal that the greatest sex differences in foetal testosterone (fT) levels are detectable between weeks 14 and 16 of gestation (5). In the amniotic fluid, the greatest sex differences have been measured between weeks 12 and 18, which is also the period when routine amniocentesis testing for chromosomal abnormalities, genetic birth defects and other conditions takes place. Finegan, Bartleman and Wong (6) proposed that the amniotic fluid, obtained from routine amniocentesis, could be used to measure prenatal hormone levels during the critical period for sexual differentiation of the brain: the variation in prenatal hormone levels could then be linked to later development of cognition and behaviour.

Animal studies demonstrate that fT plays a major role in shaping the brain as either a ‘male’ or a ‘female’ type (7). In general, these animal experiments have compared castrated males, normal males, normal females and females treated with androgens. Castrated males usually show feminized neural development, cognition and behaviour, while females treated with androgen show masculinized neural development, cognition and behaviour in a number of species (8–10).

Studies on humans exposed to abnormal hormone environments during development, as a result of genetic conditions or environmental exposure, suggest fT levels may be involved in shaping sex differences in the human brain (11, 12). One example is congenital adrenal hyperplasia (CAH), where a genetic defect of the adrenal
cortex causes overproduction of foetal androgens. Affected females show masculinization compared with their unaffected siblings (13). In addition, some sexually dimorphic behaviours are influenced by exposure to chemicals that mimic gonadal hormones (14).

**Systemizing**

Systemizing is the drive to analyze or construct systems. A recent model of psychological sex differences suggests that this is a major dimension in which the sexes differ, with males being more drawn to systemize than females (15, 16). Systemizing allows one to predict the behaviour of a system and control it. A growing body of evidence suggests that, on average, males spontaneously systemize to a greater degree than do females (17). A system is defined as something that takes inputs, which can then be operated on in variable ways, to deliver different outputs in a rule-governed way. There are at least six kinds of systems (technical, natural, abstract, social, organizable and motoric), but all share the same underlying process, which is closely monitored during systemizing (18). The systemizing quotient (SQ) was designed for adults as an instrument that could assess an individual’s interest in systems across the range of different classes (16). The present study utilizes a child version of this task (Systemizing Quotient – Children’s version, SQ-C) to examine systemizing preference in children.

**The empathizing–systemizing theory of sex differences**

A recent theory of sex differences, the empathizing–systemizing (E–S) model, proposes that two psychological dimensions are central to sex differences in the mind: empathizing and systemizing (15, 17). Empathizing is a powerful tool for understanding an individual’s behaviour and the social world, while systemizing is a powerful tool for predicting the law-governed universe. The theory postulates that, on average, females are better at empathizing than systemizing, while males are better at systemizing than empathizing. This theory proposes differences in both ability and preference, however, it does not make claims as to which may be primary. Since fT levels are known to be considerably higher in males, this study explores the sex differences in the E–S model and their relation with fT levels.

According to the E–S theory, individuals in whom empathizing is more developed than systemizing are referred to as having a type E brain. Individuals in whom systemizing is more developed than empathizing have a type S brain. Individuals, who demonstrate similar systemizing and empathizing ability are called type B (to indicate the “balanced” brain). The E–S theory states that, on average, more males than females have a brain of type S, and more females than males have a brain of type E.

Sex differences in empathizing have been found with women being better at decoding non-verbal communication, picking-up subtle nuances from tone of voice or facial expression and judging a person’s character (19). The evidence for a male advantage in systemizing includes sex differences which have been found in the general population through monitoring performance in visuo-spatial domains. An example is mental rotation, which has been studied for over 20 years and summarized in several meta-analytic reviews. The effect size, favouring males, is large (d = 0.9), according to the conventional standards of psychology, and has remained unchanged for two decades (20). The male advantage in transforming information in visuo-spatial short-term memory is seen as early as 3 years of age and in mathematical giftedness as early as 4 years (21).

A high preference for systemizing has been found in individuals with autism spectrum conditions (ASC), which are characterized by varying degrees of impairment in communication skills, social interactions, and restricted, repetitive and stereotyped patterns of behaviour (22). Individuals with this condition score higher than normal males on the SQ, who in turn score higher than normal females (16). Baron-Cohen (17) has described autism as an extreme manifestation of some sexually dimorphic traits. The extreme male brain (EMB) theory of autism (23) is an extension of the E–S model of sex differences and proposes that individuals with ASC are impaired in empathizing and are at least average or superior in systemizing, relative to their mental age (i.e. an extreme type S brain in the E–S model).

Experimental evidence supporting the EMB theory of autism includes findings that individuals diagnosed with ASC are superior to normal controls on tasks that usually give rise to male superiority, such as the embedded figures task (24). The same result has also been found in children with ASC, who used qualitatively different strategies than typically developing controls (25). In a spatial abilities task using a human-size labyrinth, individuals with high-functioning autism showed shorter map learning times and superior accuracy in graphic cued recall of a path (26). Lastly, siblings of children with autism show superior performance on spatial and verbal span tasks (27), suggesting genetic links between autism and superior spatial ability.

These results suggest that there is a biological parameter responsible for the development of increased systemizing ability and preference in males. fT levels have been identified as one such candidate (28). The mental rotation task has been found to be directly correlated with fT levels (29) as have narrow interests in children (30). Narrow interests might result from high systemizing because the interests themselves focus on specific systems, and itself entails narrow attention to details as variables playing a role in the working of the system. Both of these suggest systemizing may also be positively correlated with fT levels. If found, this would be relevant to the E–S theory, since the recent tests of
empathizing have been found to be negatively correlated with fT (30, 31). The aim of this study is to investigate the link between fT (measured in amniotic fluid) and systemizing preference in children. In addition, this study looks for sex differences in systemizing preference, given the average male advantage in various separate systemizing domains (11, 12).

**Methods**

**Participants**

Participants comprised n=204 children (93 female), age 6–9 years, taking part in a long-term study on the effects of fT (28). The mothers of these children had all undergone amniocentesis in the Cambridge region and given birth to healthy singleton infants.

**Materials**

The revised SQ-C (Fig. 1) was designed to be short, easy to complete and easy to score (Auyeung *et al.*, unpublished observations). Unlike the adult version of the SQ (16), the SQ-C was designed to be a parental-report questionnaire, rather than a self-report questionnaire, thus overcoming the problems faced in designing a questionnaire to be read and understood by children. The SQ-C is a 28-item questionnaire with four alternatives for each question. The parent indicates how strongly they agree with each statement indicating if they: (a) definitely agree, (b) slightly agree, (c) slightly disagree or (d) definitely disagree. The scoring of each item gives a value of 0, +1 or +2. A value of +2 indicates a definitely agree or disagree response (a strong empathizing or systemizing trait), a value of +1 indicates a slightly agree or disagree response (partial presence of the trait) and a value of 0 indicates the trait’s absence.

**Predictor variables**

ft levels The predictor of greatest interest in this study is fT. Testosterone in amniotic fluid was extracted with diethyl ether and measured by RIA. The ether was evaporated to dryness at room temperature and the extracted material re-dissolved in an assay buffer. The testosterone was assayed by the DPC ‘Count-a-Coat’ method (Diagnostic Products Corp., Los Angeles, CA 90045-5597, USA), which uses an antibody to testosterone coated onto propylene tubes and an $^{125}$I-labelled testosterone analogue. Research has found that, generally, dihydrotestosterone, delta-1-dehydrotestosterone and delta-1-testosterone cross-react significantly with the antibody because cross-reactions with these compounds would be expected with antibodies to testosterone conjugated at the carbon-3 position (32). The detection limit of the assay using the ether-extraction method is approximately 0.1 nmol/l. The within- and between-assay precision (coefficients of variation) have been found to be 5.2 and 6.7% respectively (32). This method measures total extractable testosterone.

The following control variables were included in our analysis.

**Gestational age at amniocentesis** Levels of fT vary during gestation. Although amniocentesis occurs around a specific timepoint, this can range from the 14th to 22nd week. Therefore, it is important to determine whether fT is related to gestational age. Gestational age at amniocentesis was obtained from hospital records. Males ($r=0.03, P>0.05$) and females ($r=-0.03, P>0.05$) showed no linear relationship between gestational age and fT. In addition, no quadratic relationship was apparent.

**Parental age** Sociodemographic variables were also included in this study. The mean maternal age was computed. It was included because women undergoing amniocentesis have a higher mean age than the general childbearing population.

**Level of education obtained by parents** The mean maternal and paternal education level was computed. Parental education level was measured according to a five-point scale: 1, no formal qualifications; 2, O level/GCSE or equivalent; 3, A level, HND or vocational qualification; 4, university degree; and 5, postgraduate qualification. Ordinary levels (0 levels) and general certificate of secondary education (GCSE) subjects are roughly equivalent to a US honours high school curriculum. The courses are divided between five groups: Languages, Sciences, Mathematics, Humanities and Social Sciences and Creative, Technical and Vocational. Advanced levels (A levels) are a General Certificate of Education, usually taken by students in the final 2 years of secondary education, after GCSEs. It is a non-compulsory qualification taken by students in the United Kingdom. Finally, Higher National Diplomas (HNDs) are an alternative route for students, who want to get a higher education without studying for a Bachelors degree. These are more vocational than degrees and can often be more practical rather than theoretical.

**Number of siblings** Information on the number of siblings was obtained because in addition to parental age and education, siblings are a significant factor in the social environment and influence the child’s development (33, 34).
Procedure

Medical records of approximately 950 patients were examined, who had undergone amniocentesis in the Cambridge region between June 1996 and June 1997. Participants were excluded if: (a) amniocentesis revealed a chromosomal abnormality; (b) the pregnancy ended in miscarriage or termination; (c) the child suffered neonatal or infant death; (d) the child suffered significant medical problems after birth; (e) there was a
Results

Mothers of children of various ages completed the SQ-C. Possible differences between the children’s ages were examined. Differences between age-groups and SQ-C scores were tested using a one-way ANOVA. No significant differences between age-groups were found (F(4, 178) = 0.9, P > 0.05). Therefore, child’s age was not included as a co-variable in subsequent analyses.

Sex differences were examined between the predictor variables using independent samples t-test (equal variances not assumed). No significant sex differences were found for any of the predictor variables except fT. Boys (mean = 0.83 ± 0.40) had significantly higher fT levels than girls (mean = 0.28 ± 0.17), t(202) = 12.58, P < 0.001, d = 1.79. In addition, the scores on the SQ-C showed significant sex differences, t(202) = 4.88, P < 0.001, d = 0.69 with boys (mean = 27.79 ± 7.64) scoring higher than girls (mean = 22.59 ± 7.53). Table 1 presents the mean, s.d. and range for each sex separately, as well as combined.

Table 1 Means, s.d. and ranges for each sex separately as well combined.

<table>
<thead>
<tr>
<th>Predictor variable</th>
<th>n</th>
<th>Mean</th>
<th>s.d.</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both sexes combined</td>
<td>204</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fT (nmol/l)</td>
<td>203</td>
<td>0.58</td>
<td>0.42</td>
<td>0.05–2.05</td>
</tr>
<tr>
<td>Gestational age at amniocentesis (weeks)</td>
<td>125</td>
<td>16.38</td>
<td>2.02</td>
<td>13.0–22.0</td>
</tr>
<tr>
<td>Maternal age at child’s birth</td>
<td>175</td>
<td>35.76</td>
<td>4.02</td>
<td>23.39–46.46</td>
</tr>
<tr>
<td>Parent education level</td>
<td>174</td>
<td>3.25</td>
<td>1.02</td>
<td>1–5</td>
</tr>
<tr>
<td>Number of siblings</td>
<td>179</td>
<td>1.28</td>
<td>0.93</td>
<td>0–5</td>
</tr>
<tr>
<td>Females only</td>
<td>93</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fT (nmol/l)</td>
<td>93</td>
<td>0.28</td>
<td>0.17</td>
<td>0.05–0.80</td>
</tr>
<tr>
<td>Gestational age at amniocentesis (weeks)</td>
<td>57</td>
<td>16.49</td>
<td>2.59</td>
<td>13.50–22.0</td>
</tr>
<tr>
<td>Maternal age at child’s birth</td>
<td>82</td>
<td>35.61</td>
<td>3.94</td>
<td>23.39–45.35</td>
</tr>
<tr>
<td>Parent education level</td>
<td>79</td>
<td>3.07</td>
<td>0.83</td>
<td>1–5</td>
</tr>
<tr>
<td>Number of siblings</td>
<td>84</td>
<td>1.30</td>
<td>0.92</td>
<td>0–5</td>
</tr>
<tr>
<td>Males only</td>
<td>111</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fT (nmol/l)</td>
<td>111</td>
<td>0.83</td>
<td>0.40</td>
<td>0.10–2.05</td>
</tr>
<tr>
<td>Gestational age at amniocentesis (weeks)</td>
<td>68</td>
<td>16.28</td>
<td>1.38</td>
<td>13.00–20.00</td>
</tr>
<tr>
<td>Maternal age at child’s birth</td>
<td>94</td>
<td>35.89</td>
<td>4.10</td>
<td>24.91–46.46</td>
</tr>
<tr>
<td>Parent education level</td>
<td>95</td>
<td>3.40</td>
<td>1.13</td>
<td>1–5</td>
</tr>
<tr>
<td>Number of siblings</td>
<td>95</td>
<td>1.26</td>
<td>0.95</td>
<td>0–5</td>
</tr>
</tbody>
</table>

fT, free testosterone.

To examine the relationship between fT and SQ-C scores, initial analyses included both sexes together. Correlations were examined between SQ-C scores and each of the predictor variables. If the relationship was significant at the P < 0.05 level, then that predictor was included in the regression analysis (35). The only predictors to meet this criterion were fT level (r = 0.38, P < 0.001) and sex (r = 0.33, P < 0.001). The presence of suppressor variables was examined by looking at any other variable found to be significantly correlated to the predictors that were included in the model at the P < 0.05 level (see Table 2 for correlations between the variables). Parental education was the only suppressor variable found and was included in the regression analysis.

Examination of SQ-C scores and fT using scatter plots suggested a linear relationship. A stepwise linear regression analysis was, therefore, conducted to find the best fit for the dependence of SQ-C scores on the predictor variables (entry criteria P < 0.05, removal criteria P > 0.1). The only predictor included in the model was fT, which significantly predicted SQ-C scores, β = 0.37, t(172) = 5.29, P < 0.01. fT also explained a significant proportion of variance in SQ-C scores, R² = 0.14, F(1, 172) = 27.98, P < 0.01 (Fig. 2).

Analyses were then conducted within each sex (using the same procedure) to investigate further the role of sex, fT and SQ-C scores correlated significantly in boys (r = 0.25, P < 0.05) and girls (r = 0.24, P < 0.05). Therefore, stepwise linear regression analyses were conducted. In the stepwise regression analysis for girls, parent education level (β = 0.23) and fT (β = 0.22) were retained in the model and accounted for a significant proportion of the variance in SQ-C scores, R² = 0.12, F(1, 76) = 5.17, P < 0.01. For boys, the only predictor included in the model was fT (β = 0.23) and this explained a significant proportion of variance in SQ-C scores, R² = 0.05, F(1, 93) = 5.29, P < 0.05 (see Table 3).

Discussion

This study examined the relationship between fT levels, measured via amniocentesis, and systemizing preference in children. The SQ-C version (Auyeung et al., unpublished observations) was used to assess systemizing preference in children. Results showed a positive association between fT levels and SQ-C score, when boys and girls were examined together. Boys also scored significantly higher than girls on the SQ-C. However, in girls, an additional predictor of parental education level was included in the final regression model. When sexes were examined together, the only significant predictor was fT. Sex was not included in the final regression model, suggesting that fT levels play a greater role than the child’s sex in terms of differences in systemizing preference.

The results from this study suggest that there is a sensitive period for the influence of hormones on brain development at around the same time when
amniocentesis is carried out (generally between weeks 14 and 22 of gestation). These results also confirm the previous research showing that fT is related to the development of behaviour and cognition (28). The results in this study differ from these previous studies in that girls have been shown to also demonstrate the relationship as well as boys. The sex differences found in this study replicate those found in adults, where men score higher than women in systemizing preference, and those with autism score even higher (16). This pattern has also been found in children (Auyeung et al., unpublished observations).

A possible explanation for the inclusion of parental education level in the final regression model for girls is that parents, who have achieved higher levels of education may influence their children by exposing them to more structured activities, enhancing their child’s preference in this area.

The finding of sex differences in systemizing is consistent with previous research showing that males and females differ in cognition and behaviour in addition to morphology and physiology (36). Within the area of cognition, the male advantage in specific aspects of spatial ability has been observed in several species, including humans (37). Sex differences in spatial ability have been found in areas, such as mental rotation and spatial perception (38). An example of mental rotation is the ability to rotate a two- or three-dimensional object rapidly and accurately (39). Spatial perception tests require participants to determine spatial relationships with respect to the orientation of their own bodies, in spite of distracting information (40).

These results suggest that fT levels are a biological predictor for systemizing preference, and may help to explain the increased occurrence of ASC in males. Previous findings have also implied that fT is a predictor of empathizing preference and ability in children (Chapman et al., unpublished observations). Levels of fT have also been found to significantly predict the number of autistic traits a child displays (41), a result consistent with the EMB theory of autism (17). Finally, it suggests that fT is a biological factor that plays a role in cognitive sex differences and is consistent with the E–S theory of sex differences.

Limitations and future directions

Difficulties are inherent in this study of foetal endocrinology. One such difficulty is that the lowest fT levels in our sample were near the detection limit of the assay (0.1 nmol/l). We investigated the distribution of scores to determine whether there was a floor effect (particularly, for girls). No girls had undetectable levels of fT. Only two girls (about 2% of the female sample) scored below 0.2 nmol/l, indicating that there was not a strong floor effect. In addition, the assay used in this study measured total extractable testosterone. Since binding proteins and degradation enzymes affect the availability of testosterone, its actual effect does not depend solely on the total extractable amount found in amniotic fluid. The presence and sensitivity of androgen receptors are also important in determining how strong the effects of testosterone are. The present study was not able to address such questions.

This study relies on maternal report which has some drawbacks, notably that mothers may interpret individual questionnaire items differently. Laboratory tests or observations should be created to test these abilities in vivo, which would allow for less subjective results. However, an advantage of maternal report is that mothers may be good judges of their children’s

Table 2 Correlation matrix showing relationships between the independent variables for all subjects of both sexes.

<table>
<thead>
<tr>
<th>SQ-C scores</th>
<th>fT (nmol/l)</th>
<th>Child’s sex</th>
<th>Gestational age</th>
<th>Parental age</th>
<th>Parental education level</th>
<th>Number of siblings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>fT (nmol/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child’s sex</td>
<td>0.38*</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age</td>
<td>–0.08</td>
<td>0.66*</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parental age</td>
<td>–0.03</td>
<td>–0.06</td>
<td>–0.07</td>
<td>–0.22†</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Parental education level</td>
<td>0.10</td>
<td>0.11</td>
<td>0.17†</td>
<td>–0.12</td>
<td>0.12</td>
<td>0.09</td>
</tr>
<tr>
<td>Number of siblings</td>
<td>–0.00</td>
<td>0.03</td>
<td>–0.02</td>
<td>–0.03</td>
<td>0.09</td>
<td>–0.07</td>
</tr>
</tbody>
</table>

Note: n varies due to missing data for some participants. Correlations are Pearson correlations. *P<0.01, †P<0.05. SQ-C, systemising quotient-children’s version; fT, free testosterone.

Figure 2 Relationship between free testosterone (fT) level and systemizing quotient-children’s version (SQ-C) scores for the combined group.
strengths and weaknesses in a variety of contexts and over an extended period of time. Parental report also allows for research with a much larger sample size than in vivo testing and/or naturalistic observation.

The results did not take into account the effect of the neonatal testosterone (nT) surge that begins within the first few days after birth, reaching its height around the third or fourth month (42), and is measurable in blood samples up to the sixth month of postnatal life (43). It is possible that nT also affects the development of systemizing ability. Ongoing research is presently investigating this.

Finally, we have assumed that there is no difference in amniotic testosterone levels in mothers, who undergo amniocentesis compared with those who do not. In this study, mothers were advised to undergo the amniocentesis procedure for clinical reasons, due to high maternal age or other factors, suggesting a risk for Down’s syndrome and related foetal abnormalities. A random sample of amniocentesis results would not be ethical to collect because of the risks involved in the procedure; approximately 1% of amniocenteses result in miscarriage (44, 45). No link was identified between nT and parental age in this study. It is also possible that the families who have participated in this study are a representative sample of the Cambridgeshire region, but may not be representative of the population as a whole.

A strength of the amniocentesis design is that it measures testosterone produced by the foetus during a period in which it is hypothesized that masculinization of the brain occurs. Some previous studies investigating that the relationship between nT and cognitive development in humans have relied on individuals with abnormal hormonal environments during pregnancy, such as those with CAH, or those exposed to drugs that mimic or block natural hormones (46–49). In these cases, it is difficult to differentiate between the effects of the hormonal environment, a genetic abnormality associated with the disorder, or any additional effects that drugs may produce. We suggest that the present sample is more representative of the general population than studies based on abnormal environments.

### Summary and conclusions

Systemizing is the drive to analyze or construct systems and a recent model of psychological sex differences suggests that this is a major dimension in which the sexes differ, with males being more drawn to systemize than females. This study examined nT levels (measured in amniotic fluid) as a candidate biological factor that influences this cognitive aspect of sex differences in systemizing in children. The SQ-C was administered to these mothers to answer on behalf of their child. Males scored significantly higher than females, suggesting that boys systemize to a greater extent than girls. Stepwise regression analysis revealed that nT was the only significant predictor of systemizing preference when the sexes were examined together. Sex was not included in the final regression model, suggesting that nT levels play a greater role than the child’s sex in terms of differences in systemizing preference. This study suggests that levels of nT are a biological factor that play a role in cognitive sex differences and lends support to the E–S theory of sex differences.

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