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The 2nd to 4th Digit Ratio and Autism

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It has been suggested that autism may arise as the result of exposure to high concentrations of prenatal testosterone. There is evidence that the ratio of the lengths of the 2nd and 4th digit (2D:4D) may be negatively correlated with prenatal testosterone. We measured the 2D:4D ratio in 95 families recruited via the National Autistic Society, U.K.. The sample included 72 children with autism (23 with Asperger's syndrome, AS), 34 siblings, 88 fathers, 88 mothers and their controls. We found that the 2D:4D ratios of children with autism, their siblings, fathers and mothers were lower than population norms. Children with AS, who share the social and communicative symptoms of autism but have normal or even superior IQ, had higher 2D:4D ratios than children with autism but lower ratios than population norms. There were positive associations between 2D:4D ratios of children with autism and the ratios of their relatives. Children with autism had lower than expected 2D:4D ratios and children with AS higher ratios than expected in relation to their father's 2D:4D ratio. We conclude that 2D:4D ratio may be a possible marker for autism which could implicate prenatal testosterone in its aetiology.

Running Title: 2nd to 4th Digit Ratio and Autism

Autism is a severe psychiatric disorder, which is heritable and manifests itself in children from birth or infancy (American Psychiatric Association, 1994). Characteristically autism is associated with an inability to form normal social relationships or normal communication. A condition with strong similarities with autism, Asperger's syndrome (AS), is associated with a pragmatically odd language. However, children with Aspergers do not have delayed language development and have normal or even superior IQ.

There is accumulating evidence that autism is caused by one or more abnormalities in the brain which result from factors such as developmental instability. Developmental instability may arise from both genetic and environmental influences. Minor physical anomalies (MPA's) arise from early fetal maldevelopment. Studies of the frequency of MPA's in children with autism, their siblings and other controls have often shown elevated frequencies of anomalies in the autistic group (Steg and Rapoport, 1975; Walker, 1976; Campbell et al, 1978; Links et al, 1980; Links, 1980; Gualtieri et al, 1982; Arrieta et al, 1993; Rodier et al, 1997).

One striking characteristic of autism is that it is strongly sex-dependent. Children with autism show a sex ratio of 4:1 (male to female) across the full IQ range (Rutter, 1978), rising to 9:1 among children with AS (Wing, 1981). Males typically excel at spatial and mathematical tasks (Halpern, 1992; Voyer et al, 1995). Students in the disciplines of mathematics, physics and engineering are more likely to have a relative with autism and fathers and grandfathers of children with autism are over-represented in occupations such as engineering (Baron-Cohen et al, 1997). Evidence such as this has been developed into the "extreme male brain theory of autism" (Baron-Cohen and Hammer, 1997). Testosterone is known to have a wide range of prenatal extra-genital effects which include an influence on the developing Central Nervous System (Geschwind and Behan, 1982). Geschwind and Galaburda

(1985) suggested that testosterone inhibited the growth of certain areas of the left hemisphere and facilitated the growth of the same areas in the right hemisphere. It is not possible to directly test the prenatal testosterone levels of children and adults. Indirect tests of the Geschwind and Galaburda hypothesis have usually sought to establish associations between left-handedness and traits such as autism. Such tests have often led to disappointing results (Bryden et al, 1994). A trait which is set in utero and which is correlated with prenatal testosterone would provide an alternative way to investigate a testosterone linked aetiology for autism.

The ratio between the length of the 2nd and 4th digit (2D:4D) may correlate with in utero testosterone because (a) it is sexually dimorphic (Baker, 1888; George, 1930; Phelps, 1952; Manning et al, 1998) with males having on average longer 4th digits relative to their 2nd digits (ie low 2D:4D) than females (who have on average higher 2D:4D) (b) the relative lengths of the digits is set before birth and probably by week 14 of pregnancy (Garn et al, 1975; Manning et al, 1998) (c) 2D:4D has been reported to be negatively correlated with testosterone and positively associated with oestrogen in adults (Manning et al, 1998) (d) the waist:hip ratio (a positive correlate of testosterone and a negative correlate of oestrogen, Evans et al, 1983) of women is negatively associated with 2D:4D ratio of their children. That is women with low waist:hip ratio (with low testosterone and high oestrogen) have children with high 2D:4D (children who are likely to have experienced low testosterone in utero; Manning et al, 1999) (e) low 2D:4D has been reported to be correlated with an increased preference to use the left hand (Manning et al, 2000).

The purpose of this work was to compare patterns of 2D:4D ratios in children with autism, asperger's syndrome, their siblings, fathers and mothers and controls.

Method

Our sample consisted of 95 participant families. The subjects included 72 children (62 boys and 10 girls) with autism (23 with normal or superior IQ i.e. high-functioning autism or Asperger's Syndrome), 34 normal siblings matched for sex with the autistic children, 88 fathers and 88 mothers. All families were members of the National Autistic Society (NAS, U.K.), and had been recruited via the NAS. Diagnosis was checked using the Autism Screening Questionnaire (ASQ, Berument et al, 1999), and all children were above cut-off. Controls were non-autistic children and adults recruited from schools and social groups and all subjects were matched for sex. There is no evidence that the 2D:4D ratios change with age (Manning et al, 1998). However, it is possible that mean 2D:4D varies between age groups as a result of differential mortality. Therefore we also matched adult subjects and controls for age.

We measured digit length from photocopies of the ventral surface of the hand. Measurements were made from the proximal crease at the base of the finger to the tip of the finger (it is known that this measurement can be made with high repeatability, Manning et al, 1998). If the basal crease of the finger was not apparent the photocopies were discarded. Vernier callipers measuring to 0.01mm were used for all measurements. The length of the 2nd and 4th digits of 30 hands was measured with callipers and also measured from photocopies (obtained from 30 different photocopiers). The repeatability or intra-class correlation coefficient (r_1, Zar, 1984) of the 2D:4D ratio (2D/4D) was high (r_1=0.89). Using repeated measures ANOVA analysis we found that the ratio (F) between the error mean squares and the groups mean squares (F=groups ms/error ms) of the 2D:4D ratios was significant (F=18.31, p=0.0001). In addition the length of the 2nd and 4th digits was measured twice from 30 photocopies. The repeatability was high (r_1=0.99) and the F ratio significant (repeated measures ANOVA, F=27.70, p=0.0001).

We concluded that the measurement error of the 2D:4D ratios was small compared to real between-subject differences in 2D:4D.

Results

There were significant correlations between the 2D:4D ratio of the left and right hands (product-moment correlation, children with autism, r=0.62, p=0.0001; siblings, r=0.57, p=0.0004, fathers of children with autism, r=0.72, p=0.0001; mothers of children with autism, r=0.70, p=0.0001). We report mean 2D:4D per individual, ie mean of left and right hands, in the following analyses (means are reported with standard errors throughout).

There were no significant sex differences between boys and girls with autism (boys, x=0.95±0.004; girls, x=0.95±0.004, unpaired t test, t=0.32, p=0.75). Table 1 shows mean values of 2D:4D ratios for index cases and controls. There were significantly lower 2D:4D ratios for (a) children with autism compared to controls (b) children with autism compared to children with AS (c) siblings of children with autism compared to controls (d) fathers of children with autism compared to controls (e) mothers with children with autism compared to controls (Figure 1). All comparisons were matched for sex with the exception of the autistic and AS comparison. A two-factor ANOVA showed no significant interaction between type of autism and sex of child indicating the higher 2D:4D ratio of children with AS was not due to sex (AS:autistic (A), F=5.93, p=0.02; male:female (B), F=0.02, p=0.90; AB, F=0.01, p=0.93).

The distribution of 2D:4D ratios of children with autism (minus AS children, n=49) and their controls is shown in Figure 2. The mean 2D:4D ratio for the autistic sample was 0.942±0.039SD and for the controls 0.987±0.036SD. The number of children in the autistic sample with a ratio of 0.987-0.039=0.948 or less was 27 (55%) and in the controls it was six (12%). This gives a measure of the degree of non-overlap of the two distributions.

There were 64 children assessed for language and for severity

of autism (ASQ score, Berument et al, 1999). The children with no language had a lower (non-significant) mean 2D:4D ratio (no language, n=51, x=0.94±0.003, language x=0.96±0.004, unpaired t test, t=1.64, p=0.11). There was a negative relationship between the ASQ (scored without language) and mean 2D:4D ratio.
However, this was not significant (b=-32.74, F=2.40, p=0.13).

There were positive relationships between the 2D:4D ratios of children with autism and their relatives. For siblings the association was non-significant (regression analysis, b=0.29, F=1.91, p=0.18). The residuals from this regression were higher for children with AS compared to children with autism (AS, 0.02±0.003; autistic, -0.01±0.003, unpaired t test, t=3.15, p=0.004). This indicated that for a given 2D:4D sibling ratio the 2D:4D ratio for children with AS was higher than expected and for children with autism lower than expected. For fathers and children with autism (including AS) the 2D:4D association was significant (n=66, b=0.41, F=7.94, p=0.006). As with siblings the residuals from the regression were higher for subjects with AS than for children with autism (AS, 0.02±0.003; autistic, -0.01±0.004, unpaired t test, t=3.15, p=0.003). This tendency for the 2D:4D ratio to be higher than expected in children with AS and lower in children with autism when compared with paternal 2D:4D was not lost when the sex of the child was controlled for (two-factor ANOVA, AS:autism (A), F=6.31, p=0.01; sex (B), F=0.02, p=0.88; AB, F=0.06, p=0.81). The relationship between 2D:4D of mothers and children with autism (including AS) was positive but non-significant (n=67, b=0.24, F=3.04, p=0.09). The residuals for children with AS from this regression were again higher than those for the children with autism (AS, 0.02±0.003; autistic, -0.01±0.003, t=2.93, p=0.005). However this effect was lost when the sex of the child was controlled for (two-factor ANOVA, AS:Autism (A), F=3.41, p=0.07; Sex (B), F=2.72, p=0.10; AB F=0.01, p=0.91). The overall impression from these correlations is that there are

associations between the 2D:4D ratios of children with autism and their relatives but that for a given 2D:4D of the relative (sibling, father or mother) the children with AS have higher than expected 2D:4D ratios and children with autism lower than expected 2D:4D ratios. A regression of the 2D:4D of children with autism (including AS) on mid-parent 2D:4D (mean parental 2D:4D) gave a significant heritability (Falconer, 1981) score of 0.58 (n=62, h^2=0.58±0.19SE, F=9.76, p=0.003).

Discussion

In comparison with controls we have found lower 2D:4D ratios in children with autism, and their siblings, fathers and mothers. This suggests that families with low 2D:4D ratios are at increased risk of autism. Within our group of children with autism, the non-AS children had lower 2D:4D ratios than the children with AS. This may mean that low 2D:4D is related to reduced probability of language acquisition and increased likelihood of mental handicap. In support of this lack of language acquisition was associated with a reduced 2D:4D ratio (non-significant) and the ASQ was negatively but non-significantly correlated with 2D:4D. The children with AS had lower 2D:4D ratios than controls but the difference was non-significant.

The intra-familial patterns of 2D:4D ratio suggested a significant positive relationship between fathers and their children with autism. The residuals from this regression showed that non-AS children had lower than expected 2D:4D in relation to their father's 2D:4D and in the case of children with AS higher 2D:4D than expected. Weaker but similar patterns were found between children with autism and their mothers and normal siblings. However, there was not a clear pattern of lowered 2D:4D in children with autism in relation to their close relatives. If 2D:4D ratio is a measure of prenatal testosterone then children with autism and their fathers appear to experience similar amounts of androgen in utero. It follows that high levels of prenatal testosterone do not invariably result in the autistic phenotype.

Our findings suggest the following model. Low 2D:4D in children is associated with an increased risk of autism. This may be because 2D:4D ratio is itself related to high prenatal testosterone, but we have no direct evidence for this. In addition to the genes or conditions which give rise to low 2D:4D ratios there may be another factor(s) which precipitates the autistic phenotype. This may be increased developmental instability. The bone-to-bone ratios of the digits are determined by the end of the 13 or 14th week of

gestation (Garn et al, 1975). Minor physical anomalies (MPA's) are a suite of markers of developmental instability which are formed at approximately the same time as the 2D:4D ratio ie in the first trimester. They include fused, curved and crooked digits and toes, ear asymmetries etc and are found in high frequencies in neurotic, learning disabled and severely disturbed children (Steg and Rapoport, 1975; Thornhill and Moller, 1997). children with autism have higher frequencies of MPA's than their normal siblings and controls (Steg and Rapoport, 1975; Walker, 1976; Campbell et al, 1978; Links et al, 1980; Links, 1980; Gualtieri et al, 1982; Arrieta et al, 1993; Rodier et al, 1997). First trimester infection with rubella may increase developmental instability and result in autistic symptoms (Chess et al, 1971). Furthermore an increased incidence of prenatal complications can also lead to autism (Knobloch and Pasamanick, 1975; Torrey et al, 1975).

Families with low 2D:4D ratios may provide further factors (particularly high prenatal testosterone) which amplify the tendency towards developing autism. Long 4th digits (relative to body size) and high developmental instability have been reported to correlate positively with intensity of depression scores in men but not women (Martin et al, 1999). In addition prenatal testosterone has been implicated in the pattern of excess males found in some aspects of immune disease, migraine and developmental learning disorder (Geschwind and Behan, 1982). A low 2D:4D ratio may therefore be a marker for male-typical characteristics across a range of dimensions. The present finding that autism is associated with a lower 2D:4D ratio is therefore compatible with the "extreme male brain" theory of autism (Baron-Cohen and Hammer, 1997).

We conclude that low 2D:4D ratio may serve as one possible marker of autism. Low 2D:4D may also implicate prenatal testosterone in the aetiology of autism, possibly for genetic reasons. This remains to be explored.

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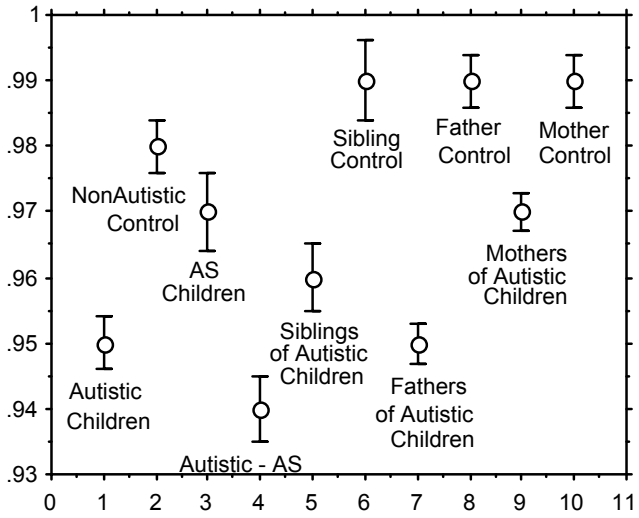
FIGURE 1

Mean 2D:4D ratios and standard error bars of the following samples: 1=children with autism, 2=normal controls; 3=children with AS, 4=children with autism with AS children removed from sample; 5=siblings of children with autism, 6=normal controls; 7=fathers of children with autism, 8=normal controls; 9=mothers of children with autism, 10=normal controls.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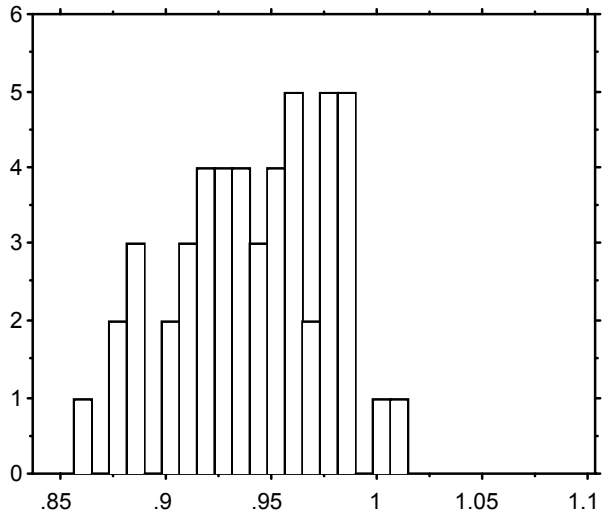
AS Children
Mothers of Autistic Children
Siblings of Autistic Children
Autistic Children
Fathers of Autistic Children
Autistic - AS

FIGURE 2

The distributions of mean of left and right hand 2D:4D ratios for children with autism (with AS children removed, n=49) and for controls.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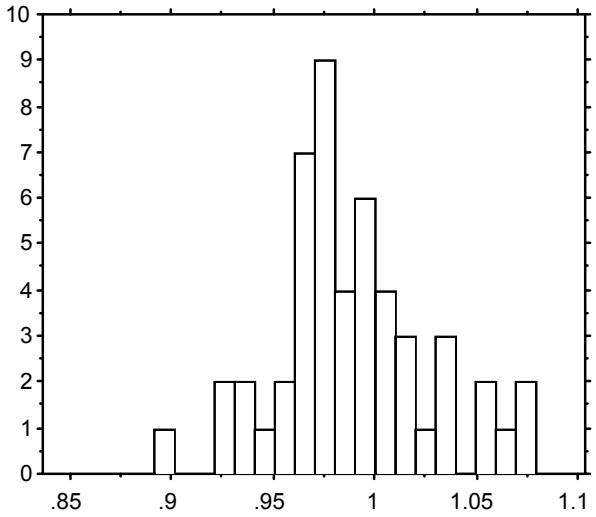
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Table 1

Comparisons of mean 2D:4D ratios from (a) children with autism and controls (b) children with autism and children with Asperger's syndrome (c) siblings of children with autism and controls (d) fathers of children with autism and controls and (e) mothers of children with autism and controls. Controls were matched for age and sex. Paired and unpaired t Tests were used. All p values are less than 0.001, therefore with Bonferroni correction for multiple tests the highest p value is 0.005.

TABLE 1

	Mean 2D:4D	Standard Error	t Test	p
Children with Autism	0.95	0.004	Paired t Test t=5.36	0.0001
Controls	0.98	0.004		
Autism Sample minus children with AS	0.94	0.005	Unpaired t Test t=3.35	0.001
Children with AS	0.97	0.006		
Siblings of Children with Autism	0.96	0.005	Paired t Test t=5.13	0.001
Controls	0.99	0.006		
Fathers of Children with Autism	0.95	0.003	Paired t Test t=6.88	0.0001
Controls	0.99	0.004		

Mothers of Children with Autism	0.97	0.003	Paired t Test t=3.27	0.0001
Controls	0.99	0.004		