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 2D:4D in 95 families recruited via
the National Autistic Society, UK. The sample comprised a
total 72 children with autism (62 males, 10 females; age range
2 to 14 years), including 23 children (20 males, three
females) with Asperger syndrome (AS), 34 siblings, 88
fathers, 88 mothers and sex- and age-matched control
participants. We found that the 2D:4D ratios of children with
autism, their siblings, fathers and mothers were lower than
population normative values. Children with AS, who share
the social and communicative symptoms of autism but have
normal or even high IQ, had higher 2D:4D ratios than children
with autism but lower ratios than population normative
values. 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 fathers' 2D:4D ratio. It was concluded that 2D:4D ratio
may be a possible marker for autism which could implicate
prenatal testosterone in its aetiology.

Autism is a pervasive developmental 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 to communicate normally. Asperger syndrome (AS), a condition with strong similarities to autism, is associated with a stilted and stereotyped use of language. However, children with AS do not have delayed language development and have normal or even high 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 (MPAs) arise from early fetal maldevelopment. Studies of the frequency of MPAs in children with autism, their siblings, and other control children have often shown elevated frequencies of anomalies in the group with autism (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). Tertiary 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 extragenital effects which include an influence on the developing CNS (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 (1) 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 (i.e. low 2D:4D) than females (who have on average higher 2D:4D); (2) 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); (3) 2D:4D has been reported to be negatively correlated with testosterone and positively associated with oestrogen in adults (Manning et al. 1998); (4) 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); (5) low 2D:4D has been reported to be correlated with an increased left hand preference (Manning et al. 2000).

The purpose of this work was to compare patterns of 2D:4D ratios in children with autism, children with AS, their siblings, fathers and mothers, and control children.

Method

Our sample consisted of 95 participant families. Participants included 72 children (62 males, 10 females; age range 2 to 15 years) with autism (20 males, three females) with normal or high IQ (i.e. high-functioning autism or AS), 34 normally developing siblings matched for sex with the children with autism, 88 fathers and 88 mothers. All families were members of the National Autistic Society (NAS, UK.), and had been recruited via the NAS. Diagnosis was checked using the Autism Screening Questionnaire (ASQ; Berument et al. 2001), and all children were above the cut-off point for autism. Control participants were children and adults without autism recruited from schools and social groups and all participants 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 and control participants 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 0.01 mm 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 intraclass correlation coefficient (r_1 Zar 1984) of the 2D:4D ratio was high ($r=0.89$). Using repeated measures ANOVA analysis we found that the ratio (F) between the groups mean squares (ms) and the error 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 ($r=0.99$) 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, i.e. mean of left and right hands, in the following analyses (means are reported with standard errors throughout).

No significant difference was found between males and females with autism (males, mean 0.95 (SD 0.004); females, mean 0.95 (SD 0.004), unpaired *t*-test, $t=0.32$, $p=0.75$). Table I shows mean values of 2D:4D ratios for index cases and control participants. There were significantly lower 2D:4D for (1) children with autism compared to control children; (2) children with autism compared to children with AS; (3) siblings of children with autism compared to control participants; (4) fathers of children with autism compared to control participants; and (5) mothers with children with autism compared to control participants (Fig. 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, $F=5.93$, $p=0.02$; male:female, $F=0.02$, $p=0.90$; AB, $F=0.01$, $p=0.93$).

The distribution of 2D:4D ratios of children with autism (minus AS children, $n=49$) and their control participants is shown in Figure 2. Mean 2D:4D ratio for the sample with autism was 0.942 (SD 0.039) and for the control participants 0.987 (SD 0.036). 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 control participants it was six (12%). This gives a measure of the degree to which the two distributions did not overlap.

There were 64 children assessed for language and for severity of autism (ASQ score; Berument et al. 1999). Children with no language had a lower (non-significant) mean 2D:4D ratio (no language, $n=51$; mean 0.94, SD 0.003; language, mean 0.96, SD 0.004; unpaired *t*-test, $t=1.64$, $p=0.11$). There was a negative relation between the ASQ (scored without language) and mean 2D:4D ratio. However, this was not

Table I: Comparisons of mean 2D:4D ratios from all groups tested

|	Mean 2D:4D	Standard error	t-test	p^a
Children with autism	0.9	0.004	Paired, 5.36	0.0001
Control participants	0.98	0.004		
Autism sample minus children with AS	0.94	0.005	Unpaired, 3.35	0.001
Children with AS	0.97	0.006		
Siblings of children with autism	0.96	0.005	Paired, 5.13	0.001
Control participants	0.99	0.006		
Fathers of children with autism	0.95	0.003	Paired, 6.88	0.0001
Control participants	0.99	0.004		
Mothers of children with autism	0.97	0.003	Paired, 3.27	0.0001
Control participants	0.99	0.004		

[a]All *p* values <0.001; with Bonferroni correction for multiple tests the highest *p*=0.005.

significant (regression analysis coefficient $b=-32.74$, $F=2.40$, $p=0.13$).

There were positive relationships between the 2D:4D ratios of children with autism and their relatives. For siblings the association was non-significant ($b=0.29$, $F=1.91$, $p=0.18$). The residuals from this regression were higher for children with AS compared to children with autism (AS, mean 0.02, SD 0.003; autistic, mean -0.01, SD 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
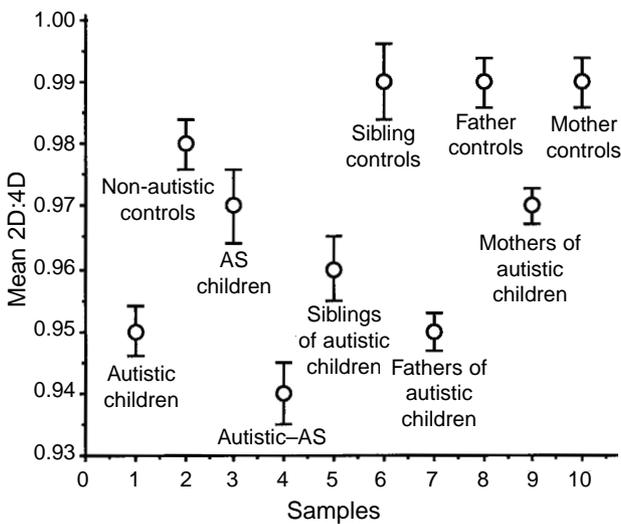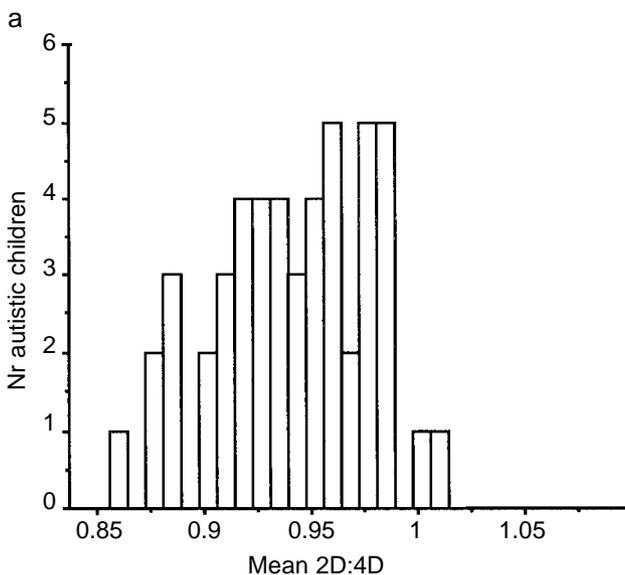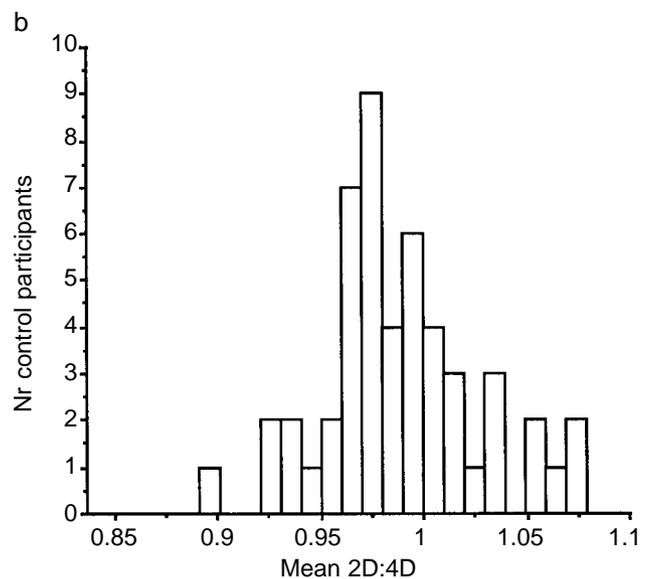
(including AS) the 2D:4D association was significant ($n=66$, $b=0.41$, $F=7.94$, $p=0.006$). As with siblings the residuals from the regression were higher for subjects with AS than for children with autism (AS, mean 0.02, SD 0.003; autistic, mean -0.01 SD 0.004; unpaired t-test, $t=3.15$, $p=0.003$). 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, $F=6.31$, $p=0.01$; male:female , $F=0.02$, $p=0.88$; AS/autism:male/female, $F=0.06$, $p=0.81$). The relation 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, mean 0.02, SD 0.003; autistic, mean -0.01 SD, 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, $F=3.41$, $p=0.07$; male:female, $F=2.72$, $p=0.10$; AS/autism:male/female, $F=0.01$, $p=0.91$). 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 a relative (sibling, father, or mother) children with AS have higher than expected 2D:4D ratios and children with autism have lower 2D:4D than expected. 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 ($n=62$, $h^2=0.58$, SE 0.19, $F=9.76$, $p=0.003$).

Discussion

In comparison with control participants we have found lower 2D:4D ratios in children with autism, and their siblings, fathers, and mothers. This may suggest that families with low 2D:4D ratios are at increased risk of autism. Within our group of children with autism, children without AS had

Figure 1: *Mean 2D:4D ratios and standard error bars of each of the ten goups tested. Autistic–AS, children with autism with AS removed from sample.*

Figure 2: *Distributions of mean of left and right hand 2D:4D ratios for children with autism (with AS children removed, n=49) and for control participants.*

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 learning disability. 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. Children with AS had lower 2D:4D ratios than control individuals but the difference was non-significant.

The intrafamilial patterns of 2D:4D ratio suggested a significant positive correlation between fathers and their children with autism. The residuals from this regression showed that children without AS had lower than expected 2D:4D in relation to their fathers' 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 normally developing 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 levels of androgen *in utero*. It follows that high levels of prenatal testosterone do not invariably result in an autistic phenotype.

Our findings suggest that 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 other factors which precipitate the autistic phenotype, e.g. increased developmental instability. The bone-to-bone ratios of the digits are determined by the end of the 13th or 14th week of gestation (Garn et al. 1975). MPAs are a suite of markers of developmental instability which are formed at approximately the same time as the 2D:4D ratio i.e. in the first trimester. They include fused, curved, and crooked digits and toes, ear asymmetries, and so on and are found in high frequencies in neurotic, severely disturbed children with learning disabilities (Steg and Rapoport 1975, Thornhill and Moller 1997). Children with autism have higher frequencies of MPAs than their normally developing siblings and control children (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 a higher proportion of males found to have 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.

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