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Low index-to-ring finger length ratio in sporadic ALS supports pre-natally defined motor neuronal vulnerability

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ABSTRACT

Background: The aetiology of apparently sporadic ALS is unknown but prenatal factors are known to influence disease development. In both men and women motor neurons require testosterone for survival and axonal regeneration after injury, and androgen insensitivity leads to a form of motor neuron degeneration in men. Reduction in the ratio of index to ring finger length (2D:4D ratio) is considered a surrogate marker for high prenatal testosterone levels in both men and women. We therefore tested the hypothesis that prenatal testosterone irrespective of gender is an independent risk factor for the development of ALS later in life, and that would be reflected in a lower 2D:4D ratio in both men and women with ALS.

Methods: Patients and unrelated control individuals attending a specialist tertiary referral centre for ALS were studied. A digital camera was used to photograph hands. Finger lengths were measured by four independent scorers blind to case-control status, and the mean 2D:4D ratio derived. Analysis was by linear regression and receiver-operator curve (ROC) analysis.

Results: Controlling for differences in sex ratio between groups, the 2D:4D ratio was lower for people with ALS (n=47) than controls (n=63) ($r = -0.25$, 2-tailed $p = 0.009$).

Conclusions: ALS patients have a lower 2D:4D ratio, consistent with higher prenatal circulating levels of testosterone, and possibly a prenatal influence of testosterone on motor neuron vulnerability in later life.

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INTRODUCTION

ALS is a neurodegenerative disease of upper and lower motor neurons resulting in relentlessly progressive weakness with death from respiratory failure. Median survival is two years from symptom onset. The aetiology of apparently sporadic ALS is unknown. Outside families with known mutations, there is currently no knowledge of the 'at risk' population for ALS, although genetic factors are known to contribute to sporadic ALS disease susceptibility¹ and athleticism has been proposed to be an environmental risk factor².

Testosterone plays an important role in the normal function of motor neurons, which express androgen receptors. A degenerative disease of motor neurons, X-linked spinobulbar muscular atrophy (Kennedy's disease), results from a trinucleotide repeat expansion in the androgen receptor gene³. There is relative sparing of the oculomotor nuclei and Onuf's nucleus⁴, which is characteristic in ALS too. Kennedy's is thought to result from a combination of toxic gain-of-function as well as a loss of normal function of the androgen receptor. Neonatal androgen exposure in rats increases the ability of cortical neurons to respond to injury in adulthood⁵, and motor neuron numbers in the spinal nucleus of the bulbocavernosus in gerbils are increased by intrauterine testosterone exposure⁶. Studies of androgen insensitivity in transgenic mouse models of Kennedy's disease crossed with testicular feminization (androgen receptor null) mice, demonstrate that androgen receptor transactivation is critical for adult neuronal health and survival and occurs in direct proportion to the sensitivity of the androgen receptor⁷.

Although there is no evidence for an association between alleles coding for larger androgen receptor trinucleotide repeats and ALS⁸, testosterone is known to mediate motor neuron survival, dendritic length⁹ and axonal regeneration after injury¹⁰, as well as being anti-apoptotic¹¹, which are all contemporary themes in the current understanding of ALS pathophysiology¹², and it is reasonable to assume the androgen receptor is required for this protection to be mediated.

Male sex is associated with higher prenatal levels of testosterone¹³ and also with an increased risk of ALS¹⁴. We hypothesized that a high prenatal testosterone level rather than male sex itself is an independent risk factor for the development of ALS in later life. There is evidence that the ratio of the index and ring finger lengths (2D:4D ratio) is sexually dimorphic because it is dependent on prenatal testosterone levels, with males having on average a lower ratio (relatively longer ring fingers)¹⁵. We therefore tested the hypothesis that prenatal testosterone, as an independent risk factor for the development of ALS later in life, would be reflected in a lower 2D:4D ratio for both men and women with ALS.

METHODS

Clinical methods

Consecutive patients and unrelated individuals attending a specialist tertiary referral centre for ALS were invited to participate. The diagnosis of ALS was made by two consultant neurologists after exclusion of other conditions. The study was ethically approved by the Joint South London and Maudsley and Institute of Psychiatry Research Ethics Committee and all participants provided written informed consent prior to inclusion.

Finger length measurements

A digital camera was used to photograph hands. Photographs were taken with the hand supinated and the fingers flattened to full extension by a sheet of Perspex with the camera placed over the center of the Perspex sheet. Digit length was measured from the basal crease of the digit to the tip using the measurement tool in Adobe Photoshop. This measurement is known to show a high degree of repeatability ¹⁶. The right hand was chosen for measurement where possible. People with faint creases and those with contractures would not be reliably measurable in this study. Rather than selecting patients for these features before entry, which might be biased and arbitrary, we invited consecutive patients to participate and then used an objective rule to exclude hands with poor measurability. Images were measured by four independent scorers (UV, JG, ZM, JS) who were blinded to case-control status. Photographs with contractures resulting in finger flexion or supination were identified by flattening and whitening of skin at the finger tips and thenar eminence by the Perspex sheet, and excluded from analysis. Those in which the measurements made by the two scorers differed by more than one standard deviation were excluded from analysis. Remaining measurements were used and the average ratio taken as the result for that subject.

Statistical methods

Digit ratio was transformed into parametric data using a ladder of powers model and analysed with a paired t-test. Analyses were repeated without outliers, i.e. ratios that lay outside one standard deviation of the normal distribution, in case of bias introduced by extreme measurements. To confirm the sex ratio between patient and control groups did not

affect the results we also performed linear regression with gender included as a covariate. SPSS was used for calculations.

RESULTS

There were 141 subjects photographed (73 patients, 68 controls). Twenty one with contractures were excluded. The remaining 120 photographs were scored. Of these, ten could not be measured by one of the four scorers. The remaining 110 ratios were used for analysis.

There were 47 cases (33 males, 70 %) and 63 controls (20 males, 32 %). There was no overall difference in 2D:4D ratio between males and females (1-tailed $p = 0.41$).

However, the 2D:4D ratio was lower for the ALS group versus controls ($p=0.007$), and in linear regression with sex as a covariate this difference was independent of the sex ratio difference between the groups. The ROC curve (Figure 1) showed that the 2D:4D ratio was a discriminator of case-control status (area under the curve = 0.70, $p = 0.0001$), with a sensitivity of 72% and specificity of 65%, a positive predictive value of 61% and negative predictive value of 71%.

DISCUSSION

This study has demonstrated that a group of ALS patients have a lower 2D:4D ratio in comparison with a control group, and crucially that this is independent of sex and so not explained by the higher proportion of males in the patient group. This finger length ratio is thought to be a marker of high prenatal testosterone levels and our results are therefore consistent with the hypothesis that testosterone levels during development modify the

subsequent risk of ALS. The digit ratio has no use as a screening tool, but the ROC curve is another way of understanding the difference between the case and control 2D:4D ratios.

One limitation of this study was the exclusion of those with contractures. We attempted to limit bias by inviting every patient to participate and then analyzing photographs for measurability using an objective measure of repeatability. Nevertheless the palms with contractures are more likely to be from cases, so the analysis could not be performed in a truly blinded fashion. There is however no reason to believe that those with contractures are more likely to have higher digit ratios.

The ratio of the index and ring finger lengths (2D:4D ratio) is sexually dimorphic¹⁷, with males having on average a lower ratio (relatively longer ring fingers)¹⁸. There was no overall effect of sex on 2D:4D ratio in this study, which might be regarded as surprising given the role of androgens in determining sexual differentiation. However, the effect of sex on 2D:4D ratio is not strong because of the large variance of the sexual dimorphism of finger lengths, and larger sample sizes than we have used are required to detect it¹⁷.

Several studies hypothesize that it is prenatal testosterone levels and sensitivity to the effects of testosterone, rather than sex, that are linked most strongly with 2D:4D ratio^{15;19}, and the use of 2D:4D in investigating the effects of prenatal androgens has been reviewed in comparison to other established methods²⁰. While direct evidence for prenatal testosterone effects on 2D:4D digit ratio is not strong, a study of 33 children who had amniocentesis and were subsequently followed, supported this interpretation showing a non-significant trend

towards a correlation with intrauterine testosterone and a significant association with the ratio of testosterone to estradiol²¹. Although another study did not find a relationship between second and third trimester testosterone and 2D:4D ratio in females²², strong indirect evidence is that women and men with congenital adrenal hyperplasia, which leads to greatly elevated androgen levels in utero, have a low 2D:4D ratio^{23;24}. Thus 2D:4D ratio is likely to be a surrogate marker for intrauterine testosterone exposure. Because high prenatal testosterone is associated with sporting ability²⁵, and to 2D:4D ratio in women¹⁶, it is conceivable that this is a mechanism by which the association between ALS and sporting prowess has been observed.^{26 27}

The 2D:4D ratio does not predict adult circulating testosterone levels²⁸ and is therefore compatible with the observation that people with ALS have low levels of free testosterone²⁹. The concept of intrauterine factors influencing later disease in adult life is now firmly established³⁰, and there is evidence that intrauterine testosterone affects neuronal function in adulthood.

High intra-uterine testosterone levels might reflect pre-existing androgen insensitivity, but our reported association between low 2D:4D ratio and ALS nonetheless supports the possibility that very early factors in embryonic development might influence adult-onset motor neuronal degeneration.

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FIGURE 1. The ROC curve for 2D:4D ratio as a predictor of ALS-control status.

The optimal discriminatory position in ROC space is the top left corner, which has 100% sensitivity and specificity. The diagonal line shows the expected plot for a random, non-discriminatory test.

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ROC Curve

