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**ARTICLE DETAILS**

| TITLE (PROVISIONAL) | Does the index-to-ring finger length ratio (2D:4D) differ in amyotrophic lateral sclerosis (ALS)? Results from an international online case-control study |
| AUTHOR | Parkin Kullmann, Jane; Pamphlett, Roger |

**VERSION 1 - REVIEW**

**REVIEWER**

| REVIEWER | Paul Wicks |
| REVIEWER | PatientsLikeMe, United Kingdom |
| REVIEW RETURNED | 24-Mar-2017 |

**GENERAL COMMENTS**

As BMJ Open has open review, it is no secret that I wrote reference 33 on the basis of the original Vivekananda paper from my collaborator Ammar Al-Chalabi's lab.

In the present study, the authors have conducted a well-designed and robust study much larger than that original one, and taking appropriate steps to mitigate bias, have clearly shown that the original results do not hold up. This is exactly the kind of important replication study we need to have in ALS research, particularly epidemiology, so we do not fall too much in love with our own ideas!

This is a very well written and well reasoned paper, with good experimental design. I have only two suggestions;

1.) I'd add the limitation that for all we know it's all those pesky people with the curled up fingers who are of real importance (though if that's the case, it's still useless as a clinical sign)

2.) I'd like the full questionnaire as an appendix because I found it time consuming to go through the website to see all the other questions, and maybe one day in a few years / decades time that website might not be live anymore.

Great study, wish I'd done it myself.

**REVIEWER**

| REVIEWER | John Manning |
| REVIEWER | A-STEM Research Group |
| REVIEWER | Swansea University |
| REVIEWER | Swansea, UK |
| REVIEW RETURNED | 13-Apr-2017 |

**GENERAL COMMENTS**

This report concerns the relationship between digit ratio (2D:4D; a
negative correlate of prenatal testosterone and a positive correlate of prenatal oestrogen) and amyotrophic lateral sclerosis (ALS). There has been one report of such a link, with ALS patients showing lower 2D:4D (higher prenatal testosterone and lower prenatal oestrogen) in comparison to controls. Both low 2D:4D and ALS have been linked to elevated physical activity and this has been suggested to support the association between low 2D:4D and ALS. The present paper used an on-line questionnaire to gather self-measured finger lengths. There were initially 949 respondents but after removal of outliers for finger measurement, problems with straightening the fingers and participants ≥40 years the authors were left with 572 participants, i.e. 202 ALS patients (125 males, 77 females) and 370 non-ALS controls (112 males, 258 females). The authors report significant sex differences in 2D:4D that were expected (male<female 2D:4D). However, there were no differences in ALS and control 2D:4D. They conclude that ALS patients do not have significantly lower 2D:4D ratios than controls. Further, they suggest that the association between ALS and exercise, if it is real, may not be explainable by a link between 2D:4D and exercise.

I think this paper is a worthwhile addition to the 2D:4D and ALS literature, which at present is sparse with one data paper and one “hypothesis” paper. The conclusion of no link between low 2D:4D and ALS indicates that the putative association needs to be considered in more detail. However, there are limitations in this study that should be noted and taken into account before further studies are designed. My comments are as follows:

1) Self-reported finger lengths – as the authors indicate these include a high rate of random errors. Experimenter-measured finger lengths generally yield mean 2D:4D’s with SD’s of about SD=.03. Even after exclusion of obvious outliers self-reported finger lengths give 2D:4D SD’s of SD = .045 to .055. This can be seen in the present data. This is a limitation, as it inevitably reduces effect sizes for relationships and indeed may cause their statistical significance to vanish altogether. For example the effect size of the sex difference in 2D:4D from experimenter measured finger length is about Cohen’s d=.5 but for the right hand in the present data it is d = .27 for controls and d = .33 for ALS patients. This needs to be discussed in the limitations.

2) Errors in the measurement of self-reported finger lengths may be greater in patients with a neurodegenerative disease such as ALS than in healthy controls. Is there any indication of this in the discarded part of the data set?

3) Am I correct in my impression that about 25% of the controls were relatives of ALS patients? Would this tend to increase similarity between patients and controls and obscure any differences? Please comment.

4) In addition to sex differences there are quite large ethnic differences in 2D:4D (low 2D:4D for East-Asian and Black individuals and high 2D:4D for Whites) and smaller differences between mean 2D:4D of nations (e.g. low 2D:4D for White Australians compared to Whites from the USA and UK). Comparisons between ALS 2D:4D and control 2D:4D do not seems to take account of these limitations. They should be made more explicit in the paper.

5) The null finding of this study deserves to be taken seriously. However, because of the above limitations any future studies would benefit from finger length measurement by trained experimenters, ideally performed directly on the fingers (not from photocopies of scans). This could be stated in the Discussion.
REVIEWER
Darnai Gergely
University of Pécs, Hungary

REVIEW RETURNED
19-Apr-2017

GENERAL COMMENTS
The present study investigated differences between 2D:4Ds in ALS and non-ALS sample. Although the authors' hypothesis was supported by a previous study conducted by Vivekananda et al., they could not confirm those findings. The findings are new, interesting and, to my knowledge, have not been reported in large sample before.
I appreciate the novelty in the manuscript. There are no methodological flaws and the paper is well-written. I have only minor suggestions:
- The figures in the manuscript are correct with adequate legends, but authors should denote significant differences in Figure 3.
- Authors correctly unfolded the limitations of the study of Vivekenenda et al. (small sample size, unbalanced gender ratio) but I think that the paper would benefit from a new paragraph in the discussion regarding the authors' recommendations to other researchers: which is the best method to measure 2D:4D ratio? do the authors encourage authors to explain their sample sizes - ideally using power analyses? do the authors think that other studies with relatively small sample sizes should be considered cautiously?

VERSİON 1 – AUTHOR RESPONSE
Reviewer 1
1) For all we know it's those people with the curled up fingers who are of real importance.
RP: This is a valid point. I have added to the limitations paragraph in the Discussion: “The results may be biased towards selecting people with early ALS, since people with advanced ALS are those who would be most likely to be unable to straighten their fingers for measurements. However, there is no evidence that the pathogenesis of ALS differs between the early and later stage of the disease, so this should not affect our conclusions”. In addition in the “Strengths and Limitations” section I have given the percentages of ALS and control subjects who could not straighten their fingers.
2) I'd like the full questionnaire as an appendix.
RP: I have included in the Methods (paragraph 1): “A PDF version of the questionnaire is available for downloading as a Supplementary Online File.”

Reviewer 2
1) Self-reported finger lengths include a high rate of random errors. This reduces effect sizes for relationships and may cause their statistical significance to vanish. This needs to be discussed in the limitations.
RP: Yes, this is true. We have added to the Discussion, in paragraph 3, the sentence: “However, the 2D:4D ratios in our study have larger standard deviations, and the gender differences of the 2D:4D ratios have smaller effect sizes, than those in an experimenter-measured finger length study (citing reference 27), which emphasises the need for large numbers of subjects in Web-based 2D:4D studies.” To emphasise this point this comment has been added to the Strengths and Limitations section: “More random errors and larger standard deviations are generally found in self-reported data. Furthermore, the limitations paragraph in the Discussion now includes: “Self-reported measurements generally have more random errors and larger standard deviations than those taken by experimenters”.
2) Errors in the measurement of self-reported finger lengths may be greater in patients with a
neurodegenerative disease such as ALS than in healthy controls.

RP: I don’t think this was of concern in our study, but in the limitations paragraph in the Discussion I’ve added the point: “Some people with ALS have cognitive deficits (reference 30 cited), and so may not have been able to measure their fingers accurately. However, it is unlikely that anybody with a clinically significant cognitive deficit would have been able to complete the extensive online questionnaire.”

3) Would having some controls who are relatives of ALS patients tend to increase similarity between patients and controls and obscure any differences?

RP: Yes, we did wonder about this, but when we compared finger ratios of controls who had family members with ALS with those who had no family members with ALS we found no differences (see Results/Sporadic and familial ALS). This lack of difference between control groups is I think good evidence that the comparisons with ALS patients remain valid. I have not made any changes to the manuscript on this point.

4) In addition to sex differences there are differences in 2D:4D and smaller differences between nations. This should be made explicit in the paper.

RP: Yes, this is a good point. At present we do not have sufficient numbers of respondents to take these factors into consideration. This point was partially made previously in the Discussion, paragraph 7 (future studies), but it is now emphasised by adding: “In addition, 2D:4Ds may be different within gene pools from people of the same ethnicity” with citing of reference 39, and by adding “and between different nations” to the sentence after this.

5) The null finding of this study deserves to be taken seriously. However, because of the above limitations any future studies would benefit from finger length measurement by trained experimenters, ideally performed directly on the fingers.

RP: Yes, this direct measurement does remain the gold standard. Reviewer 3 also asked for a comment of measurement techniques in future studies, so I have added this (penultimate) paragraph to the Discussion:

“Ideally, to be able to confidently exclude small between-group differences in 2D:4D, future studies in ALS would be of about 200 male and female ALS and control subjects (i.e. 800 subjects in all). In addition to direct experimenter measurements of finger lengths, it would be useful to photographs the hands under consistent conditions (e.g., image size, lighting, camera-finger distance), and use a computer-based measurement tool on the images, to assess inter-rater and test-retest reliability. However, undertaking such a large study in a single clinical setting would be difficult. As a rule of thumb, any study that is insufficiently powered to detect the known gender differences in 2D:4D is unlikely to be able to detect biologically significant 2D:4D differences between disease and control groups.”

Reviewer 3

1) The authors should denote significant differences in Figure 3.

RP: I think this would make the Figure a little busy, and we would then for consistency need to do the same for the other Figure. Instead, I have emphasised that p values are available in Table 2 (the source of Figure 3) by including in the Table legend: “(with p values and effect sizes)”.

2) The paper would benefit from a new paragraph in the Discussion regarding the authors’ recommendations to other researchers.

RP: This has been done (see response 5 in Reviewer 2).

I think these changes considerably improve the manuscript.
GENERAL COMMENTS

The paper is much improved and the authors have responded to almost all my suggestions. Their results do indicate caution when considering possible links between 2D:4D and ALS. However, I have one concern that the authors have not quite made clear that the major limitation of their study is the self-reported finger lengths. The 2D:4D’s calculated from this have high levels of random error. This means that the effect sizes of any relationships between 2D:4D and a target trait (in this case ALS) will be substantially weakened. The fact that they didn’t find such an association MAY be the result of the inexact nature of self-reported 2D:4D. I asked the authors to give the effect sizes for the sex differences in 2D:4D. They have done this. Although they are significant they are reduced from the expected of a d of about 0.5 to 0.6. If this is reduced the link (if it exists) between 2D:4D and ALS will be similarly reduced. A short note in the limitations section of the paper to this effect would be prudent. If (when) this study is replicated with experimenter-measured finger length this no doubt will be raised. So why not acknowledge it now.

VERSION 2 – AUTHOR RESPONSE

Reviewer 2
1) A short note in the limitations section of the paper to further explain the issue of effect sizes (with examples from the gender differences) would be prudent.
RP: The reviewer has provided us with a succinct note on this topic, so we have paraphrased this and inserted it into the limitations section as the following text:
“Nevertheles, the 2D:4D ratios in our study have larger standard deviations than those in an experimenter-measured finger length study (ref26). Our calculated 2D:4Ds therefore have relatively high levels of random error, with weakening of any relationships between 2D:4D and ALS. This can also be seen from our gender difference findings, which while significant and being close to those previously reported (ref27), had smaller effect sizes, with a reduction from d values of 0.5-0.6 in experimenter-measured studies (ref26) to 0.25-0.35 in our online study.”
In addition, the following paragraph has been moved up from later in the Discussion since it more logically follows here.

Thank you again for your reviewers’ detailed and constructive comments and suggestions on this manuscript.
Does the index-to-ring finger length ratio (2D:4D) differ in amyotrophic lateral sclerosis (ALS)? Results from an international online case–control study
Jane Alana Parkin Kullmann and Roger Pamphlett

BMJ Open 2017 7:
doi: 10.1136/bmjopen-2017-016924

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