PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Understanding autism spectrum disorder and social functioning in children with neurofibromatosis type 1: protocol for a cross-sectional multimodal study
AUTHORS	Haebich, Kristina; Pride, Natalie; Walsh, Karin; Chisholm, Anita; Rouel, Melissa; Maier, Alice; Anderson, Vicki; Barton, Belinda; Silk, Tim; Korgaonkar, Mayuresh; Seal, Marc; Lami, Francesca; Lorenzo, Jennifer; Williams, Katrina; Dabscheck, Gabriel; Rae, Caroline; Kean, Michael; North, Kathryn; Payne, Jonathan

VERSION 1 – REVIEW

REVIEWER	Esteban Vaucheret Paz Hospital Italiano de Buenos Aires Argentina Child Behavioral Neurology and Child Neuropsychology
REVIEW RETURNED	07-May-2019

GENERAL COMMENTS	Very interesting research of clinical importance. The bibliography is updated.

REVIEWER	Kuan-Lin Chen Kuan-Lin Chen, Ph.D. Associate Professor Department of Occupational Therapy; Institute of Allied Health Sciences College of Medicine National Cheng Kung University No.1 University Road Tainan City 701 Taiwan TEL:886-6-2353535 ext 5906 E-mail: klchen@mail.ncku.edu.tw
REVIEW RETURNED	10-Jun-2019

GENERAL COMMENTS	The proposed research is aimed to compare the cognition and social functioning among three groups: children with neurofibromatosis type 1 (NF1), those with autism spectrum disorder, and typically developing children. Since children with NF1 seem to have increased risk for ASD and represent a unique social cognitive phenotype unlike children with idiopathic ASD, the clinical meaning of this comparison is considered to be important with good intention. I am happy to review this study proposal and provide my comments, as follows. The research questions and aims are clearly expressed. In general, the proposed design and methodology appear to be appropriate, and the sample sizes are both realistic and adequate. Only one big issue concerns me, which is that the sample is very

	diverse. The children expected to be recruited in this study range from 3 to 15 years of age. The wide age range may make the study conclusions difficult to generalize. It is suggested that the age range be decreased or that the children be divided into several age groups. Some other comparatively minor issues that may raise concerns are as follows: (1) How the important covariates (e.g., IQ, verbal ability, ASD severity) will be controlled is not well described. If these important covariates are not well controlled, it will be unknown whether the analysis to compare the cognitive and social cognitive profiles is appropriate. (2) How these three groups of children will be matched by sex, age, and IQ is not well described. (3) Please provide the rationale for estimating the sample size. (4) What are the specific “similar guidelines” to conduct the study in the clinics? In addition, although the outcomes and statistical/analytical design appear to be explained fairly appropriately, the author has not provided discussion of the potential problems and their corresponding possible solutions. The flow of the logic of the introduction section is clear. Only a few points need to be added to make the proposal more specific. It is suggested that the current literature related to the cognitive and social cognitive profiles of the three groups, such as the comparative outcome and theoretical mechanism, be added to make the hypothesis specific.

REVIEWER	Alberto Velez-van-Meerbeke Neuroscience Research Group, Universidad Colegio Mayor de Nuestra Señora Del Rosario
REVIEW RETURNED	17-Jul-2019

GENERAL COMMENTS	It seems to me a very interesting work that can help to better characterize an important group of children with NF1 who present, in addition to learning disorders, ADHD, visuospatial diseases, features of ASD or a diagnosis of ASD. All these are risk factors that must be taken into account to establish early corrective measures, through school modifications, therapeutic management or medications. The study has well-planned objectives with a correct design and methodology. I have a single comment. Although there are few studies that establish the true prevalence of ASD in NF1, and surely the authors took the study by Richards (2015) as a reference, it is worrying that both the patients diagnosed with autism and those with autism traits are included in the sample size. In this case it would be more convenient to estimate the study by Eijk (2018) who, through a design very similar to the one being presented by the authors, finds a prevalence of 10.9% of patients in the most severe range. In this sense, the sample size that is being considered for the present study should be increased to 321 patients in order to have sufficient subjects for the analysis approach and to make the correlation with the 35 controls with a diagnosis of idiopathic ASD.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1:
No changes were requested by Reviewer 1, thank you for your comments.

Reviewer 2:
Point 1:
'Only one big issue concerns me, which is that the sample is very diverse. The children expected to be recruited in this study range from 3 to 15 years of age. The wide age range may make the study conclusions difficult to generalize. It is suggested that the age range be decreased or that the children be divided into several age groups.'

Response to Point 1:
We appreciate the reviewer's concern about the wide age range of participants. Participants with NF1 and typically developing (TD) control sample sizes have been stratified into two age groups: a younger cohort of children aged 3-5 years, and a school-aged cohort of children aged 6-15 years. As indicated in the sample size calculations, we have adequately powered the sample size of each group to accommodate the age group split. This is explained for the NF1 group in the manuscript (p. 17, para 3), but was not clear for the TD sample. To make this explicit, we have made the following edit to the sample size calculation for the TD group in the revised manuscript:

"In order to detect a d=0.65 difference between the NF1 and TD control groups on continuous outcomes, with a minimum of 85% power and a significance level of 0.05, we need to recruit at least 35 children per group, in each age cohort (e.g., younger children aged 3-5 years, and a school-aged cohort aged 6-15 years)." (p. 17, para 3)

The idiopathic ASD group will include only school-aged children.

Point 2:
How the important covariates (e.g., IQ, verbal ability, ASD severity) will be controlled is not well described. If these important covariates are not well controlled, it will be unknown whether the analysis to compare the cognitive and social cognitive profiles is appropriate.

Response to Point 2:
We appreciate the suggestion of the reviewer. The decision to covary for the effects of demographic variables may change depending on the relationship between the demographic factor (e.g., SES) and the outcome of interest. If there is a significant association between To make this clearer, the following sentence has been added to the end of the data analysis section in the revised manuscript as follows:

If particular demographic variables differed between groups (e.g., age, sex, socioeconomic status) and were related to the outcome of interest, they will be introduced as a covariate using analysis of covariance (ANCOVA). (p.16, para 2).

Point 3:
Please provide the rationale for estimating the sample size.

Response to Point 3:
We refer Reviewer 2 to pages 17 to 18 in the manuscript, where the rationale for power calculations for estimating sample sizes are described in detail.

Point 4:

What are the specific “similar guidelines” to conduct the study in the clinics?

Response to Point 4:
Thank you to the reviewer for this question. The clinics that we refer to are multi-disciplinary centers providing medical care to children with NF1. These clinics operate on similar guidelines in that they are all academic, research ready centers that are well resourced for the collection of cognitive and behavioral research data, and in some cases, neuroimaging data. We have edited this wording from the revised manuscript to avoid ambiguity as follows:

All three clinics are specialist centres for the multidisciplinary care of individuals with NF1, are well resourced for the collection of cognitive and behavioural research data, and service clinical populations thought to be representative of the wider NF1 community. (p.9, para. 1).

Point 5:
In addition, although the outcomes and statistical/analytical design appear to be explained fairly appropriately, the author has not provided discussion of the potential problems and their corresponding possible solutions.

Response to Point 5:
A potential problem with the study is that we will not find a 17-18% incidence of ASD in our NF1 sample. This issue was also raised by Reviewer 3. We refer the reader to our response to Reviewer 3’s question about the sample size of the NF1 + ASD subgroup (below), where we believe we have adequately addressed this concern. Other limitations have been outlined in the “strengths and limitations” section after the abstract.

Point 6:
The flow of the logic of the introduction section is clear. Only a few points need to be added to make the proposal more specific. It is suggested that the current literature related to the cognitive and social cognitive profiles of the three groups, such as the comparative outcome and theoretical mechanism, be added to make the hypothesis specific.

Response to Point 6:
We thank the reviewer for their suggestion. In the original manuscript, similar social cognitive deficits between idiopathic ASD and children with NF1 was highlighted (e.g., theory of mind), and we also commented on the nature of IQ in these groups (p.6, para.3,). To further elaborate on cognitive and social cognitive skills of these groups, as well as underlying theoretical constructs, the following additional edits have been incorporated:

- Comment on IQ relative to typically developing children (p.5, para.2).
- Paragraph providing an overview of social cognition in NF1, highlighting difficulties in comparison to same aged peers (p.5, para.2).
- Description of the similar factors underlying social deficits and ASD symptoms in both NF1 and idiopathic ASD, with reference to theoretical frameworks (p.6, para.2).

Reviewer 3:
Point 1:
I have a single comment. Although there are few studies that establish the true prevalence of ASD in NF1, and surely the authors took the study by Richards (2015) as a reference, it is worrying that both the patients diagnosed with autism and those with autism traits are included in the sample size. In this case it would be more convenient to estimate the study by Eijk (2018) who, through a design very similar to the one being presented by the authors, finds a prevalence of 10.9% of patients in the most severe range. In this sense, the sample size that is being considered for the present study should be

increased to 321 patients in order to have sufficient subjects for the analysis approach and to make the correlation with the 35 controls with a diagnosis of idiopathic ASD.

Response to Point 1:
We appreciate the reviewers concern regarding the nature ASD in children with NF1 in this study. Importantly, we are not only examining children with NF1 with comorbid ASD, but are examining ASD traits more broadly, in all children with NF1, to be understand the effects of mutation on autism symptoms. As part of this, we will be categorising patients with NF1 that meet diagnostic criteria for ASD. While we will recruit 200 patients with NF1 into the study, we anticipate that approximately 35 of these will have comorbid ASD (17-18%).

We are well aware of the Eijk et al (2018) paper and their estimate of a 10.9% ASD prevalence rate in a clinic based sample of 2 to 10 year old children with NF1. In the manuscript, we had referenced two previous papers, both of which returned approximately 25% prevalence estimates (Garg et al., Pediatrics, 2015; Plasschaert et al., AJMG Part B, 2015).We have now included the Eijk et al (2018) prevalence rate (p.6, para.2).

For our sample size calculations, we estimated that 17 to 18% of children with NF1 in our clinic-based sample will be diagnosed with comorbid ASD. This estimate is approximately halfway between the 11% to 25% estimate rates in these previous 3 studies. Although our estimate for the current study is higher than that of Eijk et al (2018), we are including children up to 15 years of age which we believe will capture a slightly higher number of ASD diagnoses, given a typically later recognition of ASD symptoms in NF1 (e.g., Morris et al., JAMA Psychiatry, 2016). Furthermore, our estimation is relatively conservative when compared to the Garg et al (2013) and Plasschaert et al (2015) studies. We acknowledge, however, that the number of confirmed ASD cases within our NF1 group may be less than our estimation of 17-18%, which would subsequently affect the size of our NF1 + ASD subgroup. If this occurred, we would endeavour to continue collecting participants until a subsample of 35 NF1 children with ASD was reached. The following has been added to the manuscript:

This assumes that 17-18% of children with NF1 screened as part of the study will be diagnosed with comorbid ASD, which is consistent with previous estimations.19 20 61 If the target of 35 is not met, then we will endeavour to recruit extra NF1 participants until a subgroup size is achieved. (p.17, para 3)

Once again, the authors would like to thank the Senior Assistant Editor and reviewers for the feedback and we trust that the revised manuscript has been improved. Your time in reviewing this revised submission is greatly appreciated.

VERSION 2 – REVIEW

REVIEWER	Kuan-Lin Chen Department of Occupational Therapy, College of Medicine, National Cheng Kung University, Tainan City, Taiwan (R.O.C.)
REVIEW RETURNED	16-Aug-2019

GENERAL COMMENTS	I am happy to review this study proposal. All of my concerns raised in the previous review have been responded and dealt with properly. I have no further questions. Look forward to seeing this proposal to be executed and have good results.