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# Analysis of the SNP rs3747333 and rs3747334 in NLGN4X gene in autism spectrum disorder: a meta-analysis

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## Abstract

**Background:** The SNP rs3747333 and rs3747334 in Neuroigin 4X (NLGN4X) gene have been demonstrated to be associated with the susceptibility to Autism spectrum disorder (ASDs; MIM 209850), but the results are inconsistent. Therefore, a meta-analysis of eligible studies reporting the association between rs3747333 and rs3747334 and ASD was carried out to enhance the reliability of published results.

**Methods:** A systematic literature search was performed using PubMed, Web of Science, Cochrane Library to search English articles concerning the relation between rs3747333, rs3747334 and ASD up to Sep. 21th, 2017. Summary odds ratios (OR) and 95% confidence interval (CI) were used to evaluate the risk of rs3747333, rs3747334 in the ASD. The heterogeneity and publication bias of the eligible studies were also evaluated.

**Results:** Six eligible studies involving 1284 subjects (735 patients and 549 healthy controls) were included in this meta-analysis. Overall, the results indicated that there was no significant risk elevation between rs3747333, rs3747334 variants and ASD (OR = 0.39, 95% CI 0.10–1.60). Furthermore, sensitivity analysis and publication bias analysis confirmed this result.

**Conclusions:** In conclusion, our meta-analysis suggests that the rs3747333, rs3747334 in NLGN4X gene are not frequent causes of ASD.

**Keywords:** Autism spectrum disorder, NLGN4X, rs3747333, rs3747334, A meta-analysis

## Background

### Introduction of autism spectrum disorder

Autism spectrum disorder (ASDs; MIM 209850) is a heterogeneous group of neurodevelopmental diseases defined by significant language delays, social, and communication deficits, and highly repetitive and stereotypic interest and/or behaviors [1]. The global prevalence of autism has risen to 6.2% [2]. Autism patients in China are estimated in the range of 0.6–1.8 million according to the WHO's report. According to the latest report released by Autistica, people with ASD die on average 18 years before the general population [3]. ASD is a result

of complex gene–environment interactions, with strong and clear genetic influences [4]. Although the etiologic and clinical heterogeneity are universally recognized, in practice, many studies still fail to take this into account. As of December, 2015, genetic studies have pointed to 791 potential candidate genes in ASD ([https://gene.sfari.org/autdb/HG\\_Home.do](https://gene.sfari.org/autdb/HG_Home.do)), making it difficult to find common underlying pathogenic mechanisms.

### The risk of the rs3747333 and rs3747334 in the autism spectrum disorder

Neuroigin 4X (NLGN4X) is a member of neuroligins expressed in the postsynaptic neurons and mediate transsynaptic signaling by interacting their ligand, neurexins [5]. Several studies identified significant variants in the NLGN4X gene that were replicated in two or more independent samples and were associated with

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specific phenotypes of ASD [6–8]. However, other studies found no variants in the NLGN4X gene in autistic patients in their cohorts [9–11]. To date, many common single-nucleotide polymorphisms (SNPs) of NLGN4X such as rs72413786, rs2290487, rs7049300, rs3747333, rs3747334, have been studied in extensive research on ASD. Of these, the SNP rs3747333 and rs3747334 have received more favorable observations concerning on the ASD risk [11–16]. A case–control study of Chinese descendants found that the common SNPs (rs3747333 and rs3747334) in NLGN4X gene significantly associated with risk for autism [16], a previous work showed that seven boys were carriers of mutations at three SNPs including the synonymous SNP rs7049300, the non-synonymous SNP rs3747333 (L593) and the synonymous SNP rs3747334 (L593), but the co-occurrence of rs3747333C>T leading to a substitution of leucine to phenylalanine and rs3747334C>G entails a double substitution of CTC to TTG in codon 593 and thus protect the amino acid leucine [11]. However, a work from German showed that the polymorphisms rs 3747333 and rs 3747334 did not cause any amino acid substitutions in the total of the eight detected carriers [11]. Those inconsistencies could be attributed to the phenotypic heterogeneity of ASD and to relatively small sample sizes.

### Objective

Given the increasing attention directed to the inconsistencies results, to further clarify the inconclusive association between the two SNPs and ASD, we conducted a meta-analysis with available data published up to Sep. 21th, 2017.

## Materials and methods

### Literature search and selection strategy

A systematic literature search was conducted to collect studies relevant for the current meta-analysis. First, we searched three online databases (PubMed, Web of Science, Cochrane Library) for articles published in English on associations between the rs3747333, rs3747334 in NLGN4X and ASD up to Sep. 21th, 2017. Combinations of terms identifying the gene (rs3747333, rs3747334, NLGN4X, X-linked Neuroligin 4 gene) and terms identifying the phenotype of interest (autism, autism spectrum disorder, Asperger, childhood disintegrative disorder, pervasive developmental disorder) were entered as Medical Subject Heading terms and text words. After identifying a set of publications returned from each search, we collated the results and eliminated duplicates. We then reviewed the titles, abstracts, keywords and texts of all studies to exclude studies that were clearly irrelevant. Hand-searching was carried out to determine other potential eligible studies through scanning the references

cited in the retrieved articles. Finally, we applied criteria to the set of studies to determine their inclusion or exclusion in the present meta-analysis. If the two reviewers (Hongli Sun and Ying Yang) disagreed, all the authors critically evaluated the studies to judge of the inclusion or exclusion of a certain study.

### Inclusion criteria

All the eligible articles meet the inclusion criteria:

1. The study was peer-reviewed to ensure high levels of methodological adequacy and to avoid the inevitable bias caused by dependence on investigators agreeing to provide data from unpublished studies, as suggested by the Cochrane Group [17].
2. The study was adopted as “Original Research”.
3. Assessment of the association between the rs3747333 or rs3747334 or both of them in NLGN4X and ASD.
4. Written in English.

### Data extraction

The two authors independently extracted the following data: (1) first author, (2) year of publication, (3) location, (4) predominant ethnicity of participants, (5) study design, (6) number of participants, (7) diagnostic criteria. When the study did not report sufficient data to calculate effect size, we contacted corresponding authors via e-mail to request additional information. If the contact with the author was not successful, we could not include the study in the meta-analysis.

### Statistical analysis

The association of the rs3747333, rs3747334 in NLGN4X and ASD was assessed by odds ratio (OR) with 95% confidence interval (CI). Subgroup (according to study design) analysis was evaluated by study type. Publication bias was considered present when  $P$ -value was less than 0.05. Sensitivity analysis was also carried out to evaluate the stability of the meta-analysis. Statistical heterogeneity among studies was evaluated by the Chi-square-based  $Q$ -test and the  $I^2$  index.  $I^2$  index < 50% and/or  $P$ -value > 0.10 for  $Q$ -test indicated a lack of heterogeneity among the studies. The fixed-effect model (the Mantel–Haenszel method) was utilized to calculate the pooled ORs when the heterogeneity was not significant among the studies. Otherwise, the random-effects model (the DerSimonian and Laird method) was employed. Publication bias was evaluated by the funnel plot. All statistical analyses were carried out with the STATA 12.0 (STATA Corp, College Station, Texas).

**Results**

**Meta-analyses of the rs3747333, rs3747334 in NLGN4X and ASD**

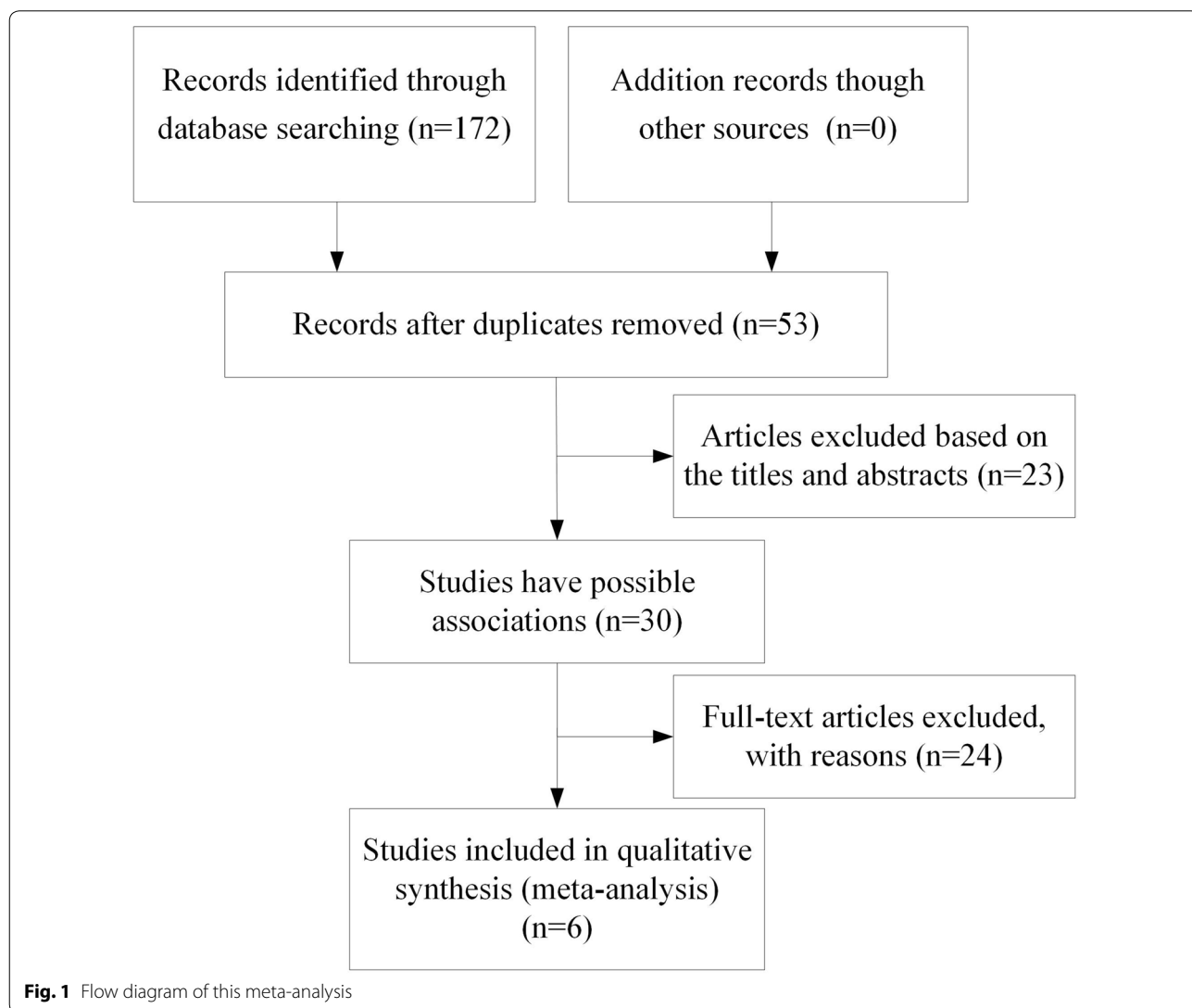
After applying inclusion and exclusion criteria (see “Materials and methods”), data from 6 studies with 1284 subjects [9, 11, 14–16, 18] were included in the meta-analysis (Fig. 1). We assessed the association between the rs3747333, rs3747334, and ASD. The characteristics of the populations used for meta-analysis are shown in Table 1.

Regarding the rs3747333, rs3747334, no statistically significant correlation with ASD (OR=0.39, 95% CI 0.10–1.60) was observed under the random-effects model. There was substantial heterogeneity in the estimated effect sizes (*I*-squared:  $I^2=95.1%$ ,  $P=0.000$ ). We further performed the subgroup analysis by study

design and the result showed that there was no statistically significant association existed between the rs3747333, rs3747334 and the risk of ASD under random-effects model (Screening: OR=0.13, 95% CI 0.05–0.31; Case-control: OR=3.68, 95% CI 0.40–33.73). There was substantial heterogeneity in the estimated effect sizes (Screening: *I*-squared:  $I^2=80.5%$ ,  $P=0.002$ ; case-control: *I*-squared:  $I^2=92.3%$ ,  $P=0.000$ ) (Fig. 2).

**Sensitivity analysis and publication bias**

The aim of sensitivity analysis was to assess the effects of each study on the pooled OR and ensure that none of solo study was completely responsible for the combined results. The results of sensitivity analysis showed that the pooled OR was not materially affected before and after removal of individual study (see Additional file 1:



**Fig. 1** Flow diagram of this meta-analysis

**Table 1 Characteristics of all studies included in the meta-analysis NLGN4X SNP rs3747333 and rs3747334**

Author, year	Ethnicity	Study design	Presence in patients/sex ratio		Presence in controls/sex ratio		Genotyping type	Diagnostic criteria	Nucleotide change
Mikhailov (2014)	Thai	Screening	9/143	5.8 M:1F	vs.dpSNP	Unknown	DHPLC <sup>a</sup>	DSM-IV <sup>d</sup>	c.1777 C>T c.17779C>G
Volaki (2013)	Greek	Screening	11/40	9 M:1F	vs.dpSNP	Unknown	PCR-DS <sup>b</sup>	DSM-IV	c.1777 C>T c.17779C>G
Wermter (2008)	German	Screening	7/107	20.4 M:1F	vs.dpSNP	Unknown	PCR-RFLP <sup>c</sup>	ICD-10 <sup>e</sup>	c.1777 C>T c.17779C>G
Blasi (2006)	Italy	Screening	4/31	3.5 M:1F	vs.dpSNP	Unknown	PCR-RFLP <sup>c</sup>	DSM-IV	c.1777 C>T c.17779C>G
Gauthier (2005)	Quebec	Case-control	61/96	6.4 M:1F	56/96	8.6 M:1F	NA	DSM-IV, ADI-R <sup>f</sup> and ADOS-G <sup>g</sup>	c.1777 C>T c.17779C>G
Xiaojuan (2014)	Chinese	Case-control	21/318	5.6 M:1F	4/453	3.0 M:1F	PCR-DS	DSM-IV and CARS <sup>h</sup>	c.1777 C>T c.17779C>G

<sup>a</sup> Denaturing high-performance liquid chromatography (DHPLC)

<sup>b</sup> Polymerase chain reaction-direct sequencing (PCR-DS)

<sup>c</sup> Polymerase chain reaction-based restriction fragment length polymorphisms (PCR-RFLP)

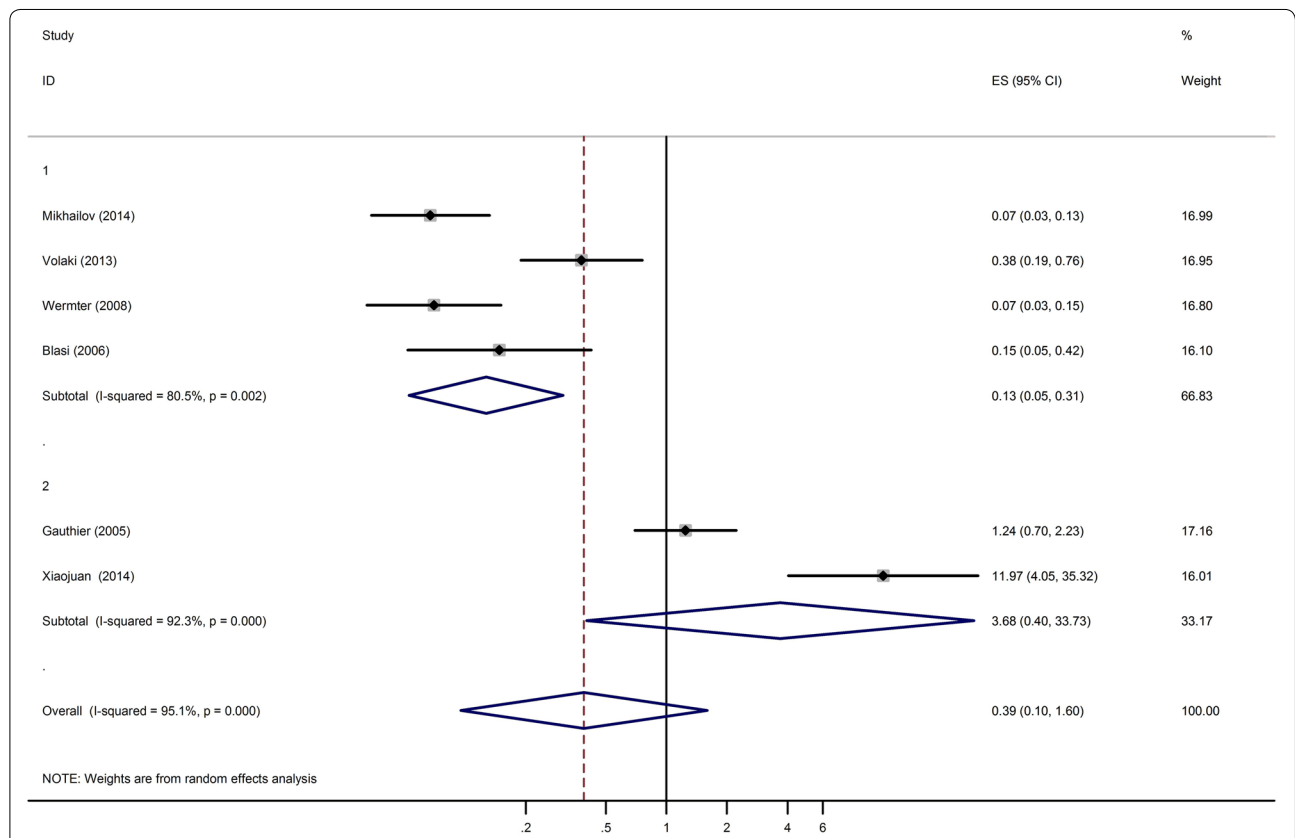
<sup>d</sup> Diagnostic and Statistical Manual of Mental Disorders, 4th ed. (DSM-IV)

<sup>e</sup> Tenth Revision of the International Classification of Diseases (ICD-10)

<sup>f</sup> Autism specific parent interview (ADI-R)

<sup>g</sup> Autism diagnostic observation scale (ADOS-G)

<sup>h</sup> Childhood autism rating scale (CARS)



**Fig. 2** Forest plot for the overall association between the rs3747333, rs3747334 variants and ASD. Each comparison is presented by the surname of the first author and the year of publication

Figures S1–S6). The studies distribution of the funnel plot was substantially symmetrical about the combined effect size in Fig. 3. Visual inspection of the funnel plot revealed obvious asymmetry.

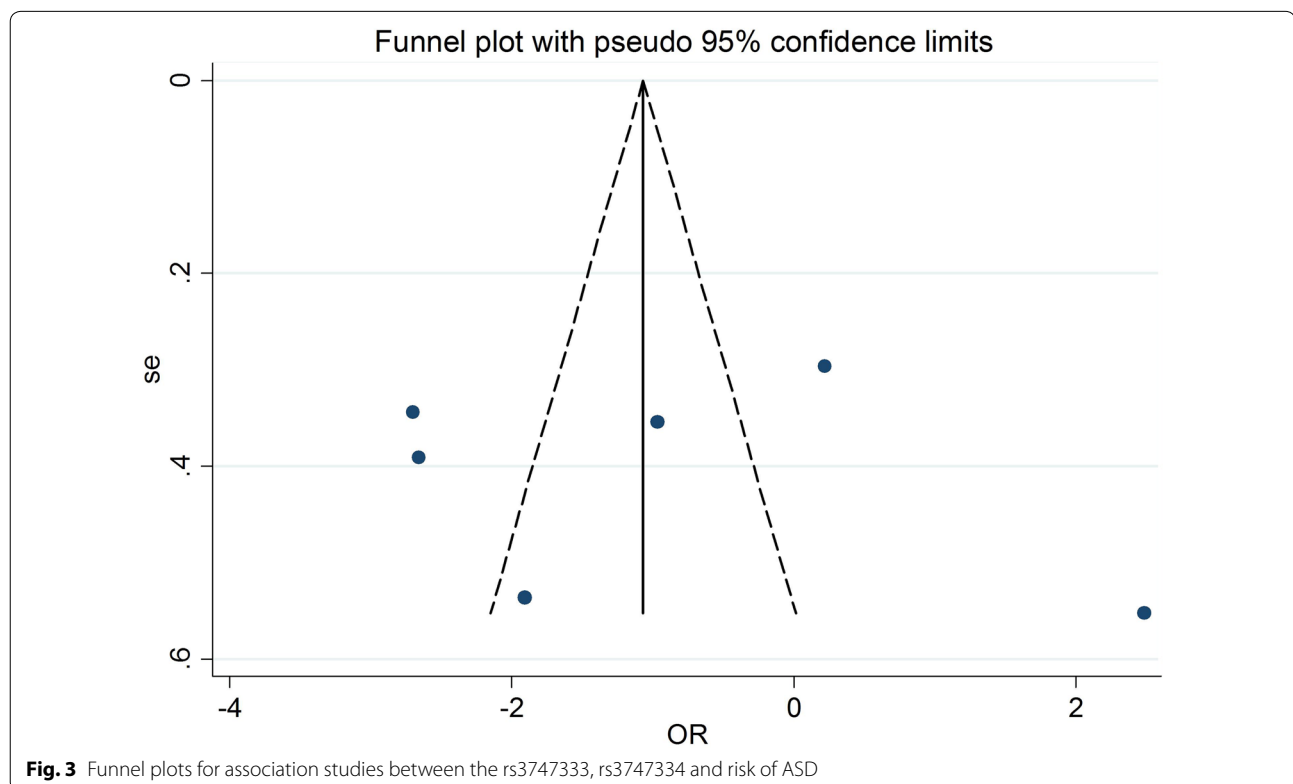
## Discussion

In the current meta-analysis, pooling the data from 6 published studies indicated the rs3747333 and rs3747334 in NLGN4X were not a risk for ASD. The rs3747333, rs3747334 are linked in exon 6 of NLGN4X, which localized on Xq13.1 and Xp22.33, encode a member of a family of neuronal cell surface proteins serving as splice site specific ligands for neurexins and may be involved in the formation, organization, and remodeling of central nervous system synapses. In our meta-analysis, the most works showed that no evidence for involvement of the rs3747333, rs3747334 in NLGN4X in probands with ASD [9, 11, 14, 15, 18], but Xiaojuan's work from Chinese sample, conversely, identified that the rs3747333 and rs3747334 in NLGN4X gene significantly associated with the risk for ASD [16]. The inconsistent results of these works are, to a very great extent, determined by ethnicity. However, it is needed to recollect more Chinese sample to identify the risk of the rs3747333, rs3747334 in NLGN4X for ASD.

The main limitation of this meta-analysis is that there was a significant heterogeneity in the estimated effect sizes across studies for the rs3747333, rs3747334 with ASD, suggesting that potential moderator variables, such as ethnicity, may cause the observed differences in effect sizes across studies. Another limitation is that we collected too few studies to reliably test moderators of that heterogeneity, and there may be some potential bias. We performed sensitivity analysis, but the results showed that the pooled OR was not altered materially when ruling out each individual study. A recent meta-analysis using genome-wide association (GWAS) analysis showed that over 16,000 individuals with highlights a novel locus at 10q24.32 and a significant overlap with schizophrenia [19], which made a great contribution to understand the complicated pathogenesis of autism spectrum disorder. Thus, genome-wide association analysis as a better approach to the study of the genetics of ASD should be applied in the future work.

## Conclusions

In summary, our meta-analysis indicates that there was no significant risk elevation between the rs3747333, rs3747334 variants and ASD based on the currently published data. However, the meta-analysis adds new information to the ongoing debate relating to the risk of



NLGN4X for ASD. Further investigations of these findings are merited by well-designed and large-sample multi-centric studies.

## Additional file

[Additional file 1.](#) Supplementary material.

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## Authors' contributions

HS was the principal investigator, participated in the study design, manuscript writing, data collecting, and analysis. YY, LZ, HW, HZ, HL participated in the study design, data collection, and writing the manuscript. All authors read and approved the final manuscript.

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## Availability of data and materials

Not applicable.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

The authors provide consent for publication.

## Competing interests

The authors declare that they have no competing interests.

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