The dopamine β-hydroxylase -1021C/T polymorphism is associated with the risk of Alzheimer’s disease in the Epistasis Project

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Abstract

Background: The loss of noradrenergic neurones of the locus coeruleus is a major feature of Alzheimer’s disease (AD). Dopamine β-hydroxylase (DBH) catalyses the conversion of dopamine to noradrenaline. Interactions have been reported between the low-activity -1021T allele (rs1611115) of DBH and polymorphisms of the pro-inflammatory cytokine genes, IL1A and IL6, contributing to the risk of AD. We therefore examined the associations with AD of the DBH -1021T allele and of the above interactions in the Epistasis Project, with 1757 cases of AD and 6294 elderly controls.

Methods: We genotyped eight single nucleotide polymorphisms (SNPs) in the three genes, DBH, IL1A and IL6. We used logistic regression models and synergy factor analysis to examine potential interactions and associations with AD.

Results: We found that the presence of the -1021T allele was associated with AD: odds ratio = 1.2 (95% confidence interval: 1.06-1.4, p = 0.005). This association was nearly restricted to men < 75 years old: odds ratio = 2.2 (1.4-3.3, 0.0004). We also found an interaction between the presence of DBH -1021T and the -889TT genotype (rs1800587) of IL1A: synergy factor = 1.9 (1.2-3.1, 0.005). All these results were consistent between North Europe and North Spain.

Conclusions: Extensive, previous evidence (reviewed here) indicates an important role for noradrenaline in the control of inflammation in the brain. Thus, the -1021T allele with presumed low activity may be associated with misregulation of inflammation, which could contribute to the onset of AD. We suggest that such misregulation is the predominant mechanism of the association we report here.

Background

Noradrenergic neurones in Alzheimer’s disease

The loss of noradrenergic neurones of the locus coeruleus is a striking feature of sporadic Alzheimer’s disease (AD). A gradual, moderate loss is found with ageing in healthy people [1-3], but a more dramatic loss is seen in AD. A meta-analysis [4] showed similarly high losses of noradrenergic neurones (24 studies) as of cholinergic neurones (33 studies), with losses four times greater than those of dopaminergic cells in AD. Noradrenergic neurones project from the brainstem to innervate wide areas of the forebrain [5]. Levels of noradrenaline (NA, norepinephrine) in target regions have also sometimes been reported lowered in ageing [6,7], e.g. in the hippocampus and hypothalamus. They have generally been found to be further reduced in AD [8-13], e.g. in the hippocampus, hypothalamus, caudate nucleus, putamen and neocortex.
although not in one small study [14]. Both the loss of noradrenergic neurones [15] and that of NA in target regions [8,13,16] have been correlated with the severity of the disease. Changes in the noradrenergic system in AD are reviewed in Hermann et al 2004 [17].

**Dopamine β-hydroxylase -1021C/T**

Dopamine β-hydroxylase (DBH) catalyses the conversion of dopamine to NA. Its activity is also reduced in post-mortem hippocampus and neocortex in AD [18,19], without correlating with the loss of noradrenergic neurones [19]. Variation in DBH activity both in serum and in CSF has been reported to be over 80% heritable [20]. The single nucleotide polymorphism (SNP), -1021C/T (rs1611115), has been identified as the main predictor of DBH activity in plasma [21,22]. It is responsible for ~30% to ~50% of the considerable variation in such activity between people, as replicated in several different populations [21,23-27]. The -1021T allele contributes to greatly lowered DBH activity through codominant inheritance [21]. In view of the chronic inflammation seen in the AD brain [28,29] and of the anti-inflammatory role of NA [30], Mateo et al 2006 [31] investigated interactions between the -1021T allele and SNPs of the regulatory regions of the pro-inflammatory cytokine genes, IL1A and IL6. They reported interactions between DBH -1021TT and both IL1A -889T (rs1800587) and IL6 -174GG (rs1800795). In the Epistasis Project, we recently confirmed [32] reported interactions between the inflammation-related cytokine genes, IL6 and IL10, that contribute to the development of AD. We therefore now decided also to examine the interactions between DBH and both IL1A and IL6 in the Epistasis Project, with 1757 cases of AD and 6294 controls. In view of the age and sex differences that have been reported in brain inflammation in the elderly [33], and of the relevant influence of sex steroids [34], we also examined possible interactions of DBH with age and sex. We found an association of the low-activity DBH -1021T allele with the risk of AD.

**Methods**

**Study population**

The Epistasis Project aims primarily to replicate interactions that have been reported to affect the risk of AD. Sample-sets were drawn from narrow geographical regions with relatively homogeneous, Caucasian populations, by seven AD research groups: Bonn, Bristol, Nottingham, OPTIMA (Oxford), Oviedo, Rotterdam and Santander. Sample characteristics by geographical region are given in Additional file 1: Table S1. All AD cases were diagnosed “definite” or “probable” by CERAD or NINCDS-ADRDA criteria. AD cases were sporadic, i.e. possible autosomal dominant cases were excluded, based on family history. The median ages (interquartile ranges) of AD cases were 79.0 (73.0-85.2) and of controls were 76.9 (71.3-83.0). Fuller details of our sample-sets are given elsewhere [32]. Ethical approval was obtained by each of the participating groups (Additional file 1: Table S2).

**Genotyping**

Blood samples were taken after written informed consent had been obtained from the subjects or their representatives. Genotyping for the six centres other than Rotterdam (below) was performed at the Wellcome Trust Sanger Institute, using the iPLEX Gold assay (Sequenom Inc.). Whole genome amplified DNA was used for 82% of samples; genomic DNA was used for the 18% of samples that were not suitable for whole genome amplification. A Sequenom iPLEX, designed for quality control purposes, was used to assess genotype concordance between genomic and whole genome amplified DNA for 168 individuals. Assays for all SNPs were designed using the eXTEND suite and MassARRAY Assay Design software version 3.1 (Sequenom Inc.). Samples were amplified in multiplexed PCR reactions before allele specific extension. Allelic discrimination was obtained by analysis with a MassARRAY Analyzer Compact mass spectrometer. Genotypes were automatically assigned and manually confirmed using MassArray TyperAnalyzer software version 4.0 (Sequenom Inc.). Gender markers were included in all iPLEX assays as a quality control metric for confirmation of plate/sample identity. Genotyping of DBH intron 10 A/G (rs1611131) and IL6 intron 2 A/G (rs2069837) was carried out using KASPar technology by KBioscience http://www.kbioscience.co.uk. No SNPs were imputed.

Genotyping in the Rotterdam cohort was done on Version 3 Illumina-Infinium-II HumanHap550 SNP array (Illumina, San Diego, USA) and additionally, SNPs were imputed using MACH software http://www.sph.umich.edu/csg/abecasis/MACH/ with HapMap CEU Release 22 as a reference [35]. The reliability of imputation was estimated for each imputed SNP with the ratio of expected and observed dosage variance (O/E ratio). Only samples with high-quality extracted DNA were genotyped; 5974 were available with good quality genotyping data; 5502 of these had reliable phenotypes. For this study, DBH exon 3 Ala197Thr (rs5320), IL1A exon 5 Ala114Ser (rs17561) and IL6 intron 2 A/G (rs2069837) were genotyped, and the other SNPs (Table 1) were imputed.

**Statistical analysis**

We assessed associations with logistic regression models, controlling for age, gender, study centre and the ε4 allele of apolipoprotein E (APOEε4), using R Version 2.10.1 (R Foundation for Statistical Computing, Vienna, Austria). The adjusted synergy factors [36] were derived from the
Table 1 Studied SNPs

| Gene | SNP       | Minor allele frequency in controls | Linkage disequilibrium in controls |
|------|-----------|------------------------------------|------------------------------------|
|      |           | North Europe | North Spain | Difference (p) | With | North Europe | North Spain |
|      |           | 20.7% (T) | 19.7% (T) | 0.47 | rs5320 | 0.994 | 0.015 | 0.994 | 0.017 |
|      | rs1611115 | -1021C/T | 5.3% (A, Thr) | 6.0% (A, Thr) | 0.38 | rs1611131 | 0.257 | 0.002 | 0.393 | 0.003 |
|      | rs 5320   | Exon 3 Ala197Thr | 29.4% (G) | 24.5% (G) | 0.002 | rs1611115 | 0.295 | 0.055 | 0.250 | 0.047 |
|      | rs1611131 | Intron 10 A/G | 29.2% (T) | 25.4% (T) | 0.01 | rs17561 | 0.999 | 0.994 | 0.989 | 0.971 |
|      | IL6       | rs1800587 | 29.2% (T) | 25.4% (T) | 0.02 | rs17561 | 0.997 | 0.185 | 0.999 | 0.145 |
|      | IL1A      | rs1800587 | 29.2% (T) | 25.4% (T) | 0.02 | rs3783550 | 0.994 | 0.185 | 0.999 | 0.144 |
|      |           | -889C/T | rs3783550 | 31.2% (C) | 29.8% (C) | 0.39 | rs1800587 | 0.994 | 0.185 | 0.999 | 0.144 |
|      |           | rs17561 | Exon 5 Ala114Ser | 31.2% (C) | 29.8% (C) | 0.39 | rs1800587 | 0.994 | 0.185 | 0.999 | 0.144 |
|      | rs3783550 | Intron 6 A/C | 31.2% (C) | 29.8% (C) | 0.39 | rs1800587 | 0.994 | 0.185 | 0.999 | 0.144 |
|      | IL6       | rs1800795 | 31.2% (C) | 29.8% (C) | 0.39 | rs1800587 | 0.994 | 0.185 | 0.999 | 0.144 |
|      |           | -174G/C | rs2069837 | 7.3% (G) | 9.3% (G) | 0.03 | rs2069837 | 0.999 | 0.055 | 0.998 | 0.049 |
|      | rs2069837 | Intron 2 A/G | 7.3% (G) | 9.3% (G) | 0.03 | rs2069837 | 0.999 | 0.055 | 0.998 | 0.049 |

SNP = single nucleotide polymorphism, DBH = dopamine β-hydroxylase, IL1A = interleukin-1α, IL6 = interleukin-6, D' = ratio of observed linkage disequilibrium to maximum possible linkage disequilibrium, r² = correlation coefficient.

Results

The data

Table 1 shows the allelic frequencies and patterns of linkage disequilibrium of the eight studied SNPs in controls. There were differences between North Europe and North Spain in allelic frequencies of five SNPs. IL1A -889C/T and exon 5 Ala114Ser were in almost 100% linkage disequilibrium. Genotype distributions of the eight SNPs in AD and controls from each of the seven centres are shown in Additional file 1: Table S3; allelic frequencies by country are given in Additional file 1: Table S4. Hardy-Weinberg analysis was performed for both cases and controls, both in the Rotterdam samples and in the samples from the other six centres, which were genotyped by the Sanger Institute. In three of these 32 analyses, the samples were not in Hardy-Weinberg equilibrium, compared with two as would be expected by chance. Those three sample-sets were all AD cases from the six centres: IL1A -889C/T (p = 0.03) and intron 6 A/C (p = 0.004), and IL6 -174G/C (p = 0.02). Since another
SNP, Arg535Cys in exon 11 of DBH (rs6271), has also been reported to influence plasma DBH activity [23,24], although much less so than -1021C/T, we performed preliminary analysis of that SNP on data from six centres, i.e. excluding Rotterdam.

**Associations of DBH -1021TT+TC with AD**

DBH -1021TT+TC versus CC was associated with AD overall: odds ratio = 1.2 (95% confidence interval: 1.06-1.4, \( p = 0.005 \)). There were interactions with sex and age (Table 2). The interaction with sex was significant overall and in North Europe, while that with age was significant overall and in North Spain. In view of those interactions, we stratified our analyses by age and by sex. Those stratified analyses established that the observed association of DBH -1021TT+TC with AD in the population was due to an association nearly restricted to men < 75 years old: odds ratio = 2.2 (1.4-3.3, \( p = 0.0004 \)) (Table 3). Similar results were obtained in North Europe and North Spain (Table 4). The DBH -1021T allele was not associated with onset age of AD.

**Interactions with IL1A and IL6**

We found an interaction between DBH -1021TT+TC and IL1A -889TT (Table 5): synergy factor = 1.9 (1.2-3.1, \( p = 0.005 \)). This interaction was consistent between North Europe and North Spain. We also found a possible interaction between DBH -1021TT+TC and IL6 -174GG (Table 5), but only in North Europe: synergy factor = 1.5 (1.07-2.0, 0.02) (Table 5). We also analysed the results for DBH -1021TT+TC and IL1A -889TT when stratified by each other (Table 6). Those analyses showed that each risk factor was only associated with AD in the presence of the other factor.

**Other DBH SNPs: exon 3 Ala197Thr (rs5320), intron 10 A/G (rs1611131) and exon 11 Arg535Cys (rs6271)**

There were no main effects of any of these SNPs. The overall odds ratio for 197Ala homozygotes (versus carriers of one or two copies of Thr) was 1.01 (0.8-1.25, 0.9) and for intron 10 AA (versus AG+GG) was 0.97 (0.85-1.1, 0.7). However, the interaction of 197Ala homozygotes with sex was slightly stronger than that of -1021TT+TC, but only in Northern Europeans: synergy factor = 2.3 (1.4-3.9, 0.001). The only apparently significant result for intron 10 AA was an interaction with age, only in Northern Spanish, very similar to that of -1021TT+TC: synergy factor = 2.1 (1.1-3.95, 0.025). The only apparently significant result in the preliminary analysis of Arg535Cys was probably due to chance (data not shown).

**Discussion**

**Interpretation of results**

We have shown a clear association between the presence of the DBH -1021TT allele and AD (Table 4): odds ratio for -1021TT+TC versus CC = 1.2 (1.06-1.4, \( p = 0.005 \)), controlling for centre, age, sex and APOE ε4 genotype. This association was nearly restricted to men < 75 years old: 2.2 (1.4-3.3, 0.0004). The interactions with sex and age were both significant (\( p = 0.01 \) and 0.03, respectively, 0.0001).

| Subset                  | Adjusted* odds ratios of AD (95% CI, \( p \)) |
|-------------------------|---------------------------------------------|
| Men                     | 1.6 (1.2-2.0, 0.0002)                        |
| Women                   | 1.05 (0.9-1.2, 0.60)                         |
| All < 75 years          | 1.6 (1.2-2.2, 0.001)                         |
| All > 75 years          | 1.06 (0.9-1.3, 0.47)                         |
| Men < 75 years          | 2.2 (1.4-3.3, 0.0004)                        |
| Men > 75 years          | 1.35 (0.98-1.8, 0.06)                        |
| Women < 75 years        | 1.3 (0.9-1.9, 0.24)                          |
| Women > 75 years        | 0.95 (0.8-1.2, 0.66)                         |

AD = Alzheimer's disease, DBH = dopamine B hydroxylase, CI = confidence interval.

* All analyses controlled for centre, age, sex and genotype of apolipoprotein E ε4.

Results in bold are significant at \( p < 0.05 \).
Table 4 Odds ratios of AD for DBH -1021TT+TC vs CC in certain subsets

| Subset            | Adjusted* odds ratios of AD (95% CI, p) |
|-------------------|----------------------------------------|
|                   | All                                    | North Europe                          | North Spain                          |
| All               | 1.2 (1.06-1.4, 0.005)                   | 1.2 (1.05-1.4, 0.01)                   | 1.3 (0.97-1.7, 0.08)                  |
| Men               | 1.6 (1.2-2.0, 0.0002)                  | 1.7 (1.3-2.2, 0.0002)                 | 1.5 (0.9-2.55, 0.12)                 |
| All < 75 years    | 1.6 (1.2-2.2, 0.001)                   | 1.55 (1.1-2.2, 0.02)                  | 1.8 (1.04-3.0, 0.03)                  |
| Men < 75 years    | 2.2 (1.4-3.3, 0.0004)                  | 2.2 (1.3-3.8, 0.002)                  | 1.9 (0.8-4.4, 0.12)                  |

AD = Alzheimer’s disease, DBH = dopamine β-hydroxylase, CI = confidence interval.
* All analyses controlled for centre, age, sex and genotype of apolipoprotein E.
Results in bold are significant at p < 0.05.

Table 5 Interactions of DBH -1021TT+TC vs CC with variants of IL1A and IL6 in AD risk

| Interaction with | Dataset | Numbers | Power* | Adjusted synergy factor (95% CI, p) |
|------------------|---------|---------|--------|------------------------------------|
| IL1A -889TT      | All     | 6137    | 1535   | 93%                                |
| vs TC+CC         |         |         |        | 1.9 (1.2-3.1, 0.005)               |
|                  | North Europe | 5678    | 1078   | 87%                                |
|                  |          |         |        | 1.7 (1.02-2.8, 0.04)               |
|                  | North Spain | 459    | 457    | 32%                                |
|                  |          |         |        | 3.4 (0.9-12.3, 0.07)               |
| IL6 -174GG       | All     | 6161    | 1565   | 95%                                |
| vs GC+CC         |         |         |        | 1.3 (0.98-1.7, 0.07)               |
|                  | North Europe | 5692    | 1084   | 88%                                |
|                  |          |         |        | 1.5 (1.07-2.0, 0.02)               |
|                  | North Spain | 469    | 481    | 44%                                |
|                  |          |         |        | 0.94 (0.5-1.7, 0.85)               |

The first column indicates the models used to represent the SNPs, IL1A -889T/C and IL6 -174G/C, in the analyses of interactions with DBH -1021C/T.
AD = Alzheimer’s disease, DBH = dopamine β-hydroxylase, CI = confidence interval.
* Power to detect a synergy factor of 1.9 (first interaction) or 1.5 (second interaction) at p < 0.05.
† All analyses controlled for centre, age, sex and genotype of apolipoprotein E.
Results in bold are significant at p < 0.05.
Table 6 Odds ratios of AD for the DBH and IL1A variants*, when stratified by each other

| Odds ratio of AD for: | In the subset: | Numbers | Controls | AD |
|----------------------|---------------|---------|----------|----|
| DBH -1021TT+TC vs CC | IL1A -889TC+CC | CC: 346 | CC: 862  | 1.1 (0.99-1.3, 0.07) |
|                      | IL1A -889TT   | TT+TC: 2077 | TT+TC: 516 |
|                      |               | CC: 340 | CC: 87   |
|                      |               | TT+TC: 174 | TT+TC: 70 |
| IL1A -889TT vs TC+CC | DBH -1021CC   | TC+CC: 3546 | TC+CC: 862 |
|                      |               | TT: 340 | TT: 87   |
|                      |               | TT: 174 | TT: 70   |
| DBH -1021TT+TC vs TC+CC |              | TC+CC: 2077 | TC+CC: 516 |
|                      |               | TT: 340 | TT: 87   |
|                      |               | TT: 174 | TT: 70   |

AD = Alzheimer’s disease, DBH = dopamine β-hydroxylase, IL1A = interleukin-1α, CI = confidence interval.
* DBH -1021TT+TC vs CC and IL1A -889TT vs TC+CC.
† All analyses controlled for centre, age, sex and genotype of apolipoprotein E.
Results in bold are significant at \( p < 0.05 \).

[8-13], raised levels of dopamine have generally not been found [8,12,13]. We will therefore base the discussion below on the hypothesis that the association of the -1021T allele with AD risk is mainly due to an effect on NA levels in the brain.

The control of inflammation in the brain

One likely result of changed DBH activity is misregulation of inflammation in the brain. The mechanisms that control inflammation in the brain differ from those in the periphery; an important part of the former control system is the noradrenergic network (reviewed in [30]). The anti-inflammatory role of NA has been shown in cultured cells and rodent brains (reviewed in [30]). Raised levels of NA reduced activation of astrocytes [46] and microglia [47-49], and lowered expression of pro-inflammatory cytokines [47-50] and chemokines [50]. Noradrenergic depletion increased production of pro-inflammatory cytokines [51] and chemokines [52], and activation of astrocytes [53] and microglia [51], and impaired microglial phagocytosis of β-amylloid [50]. Astrocytes are considered major targets of noradrenaline in the brain (reviewed in [54,55]), through their β2-adrenoceptors [46,54], which activate the cyclic AMP pathway [54,56], which may lead to the activation of peroxisome proliferator-activated receptors (PPARs) [56-58]. These receptors down-regulate expression of pro-inflammatory genes (PPARγ: [59]; PPARδ: [60]). The importance of the cyclic AMP pathway in AD was underlined by the recent identification of the cyclic AMP-response element-binding protein as the transcription factor of most relevance to networks of AD-related genes [61]. The inhibition of the pro-inflammatory transcription factor, nuclear factor-κB, by its endogenous inhibitor, IκB, may also mediate the anti-inflammatory effects of NA [62-64]. However, the anti-inflammatory role of NA remains controversial [53] and it may even have pro-inflammatory actions in certain conditions [65-67]. Nevertheless, the predominant evidence suggests a mainly anti-inflammatory, regulatory role of NA in the brain. This role is weakened in ageing [1-3] and seriously disrupted in AD [4]. Thus, elderly non-demented carriers of the DBH -1021T allele with presumed low activity may be more vulnerable to low-grade inflammation in the brain. This effect has been reported to be stronger in elderly men < 80 years old [33], consistent with our results.

Other potential mechanisms

In cell cultures and rodent brains, brain-derived neurotrophic factor (BDNF) has been reported: to be induced by NA in astrocytes and neurones [68-71]; to exert certain neuroprotective actions (reviewed in [72]); and to promote synaptic plasticity and contribute to learning and memory (reviewed in [73]). BDNF levels have been found to be decreased in the postmortem hippocampus and neocortex [74-76] in AD. A large recent meta-analysis of the BDNF Val66Met polymorphism [77] found that the Met allele was associated with AD in women, but not men.

Noradrenergic neurones also produce and secrete other neuromodulators and neurotrophins (reviewed in [78]). These neurones also have roles in glial energy metabolism [54,55] and the maintenance of the microvasculature [79,80] and of the blood-brain barrier [81]. NA has actions against oxidative stress [57,82,83] and against excitotoxicity [84,85]. Downstream of NA, the cyclic AMP pathway has neuroprotective and antioxidant actions in neuronal cultures [86,87]. NA protects against the neurotoxicity of β-amylloid (reviewed in [88]). However, potentially pathogenic contributions of NA to AD have also been reported [65,67,89].

Conclusions

Our results support an association of the functional DBH -1021T allele with increased risk of AD in men
< 75 years. Any of the above neuroprotective effects of NA (reviewed in [90]) may influence that risk and that association. However, there is considerable evidence for the role of NA in the control of inflammation in the brain (reviewed in [30]). In view therefore also of the likely interaction between DBH and the pro-inflammatory gene, IL1A, we suggest that the predominant, although not sole, mechanism of the above association with AD is misregulation of inflammation in the brain. There is substantial evidence that inflammation is an early, pre-clinical factor in the development of AD (reviewed in [91]). We have previously proposed [32] that inflammation is not only a reaction to the pathology of AD, but contributes to its onset. Our present results support that view.

Additional material

Additional file 1: Combarros et al 2010: The dopamine β-hydroxylase -1021C/T polymorphism is associated with the risk of Alzheimer's disease in the Epistasis Project

Abbreviations

AD: Alzheimer’s disease; APOEε4: apolipoprotein E ε4; CERAD: Consortium to Establish a Registry for Alzheimer’s Disease; CI: confidence interval; CSF: cerebrospinal fluid; DBH: dopamine β-hydroxylase; DBH: the gene for DBH; IL1A: the gene for interleukin-1α; IL6: the gene for interleukin-6; NINCDS-ADRDA: National Institute of Neurological, Communicative Diseases and Stroke-Alzheimer’s Disease and Related Diseases Association; OPTIMA: the Oxford Project to Investigate Memory and Ageing; SNP: single nucleotide polymorphism.

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Authors’ contributions

All authors contributed to the design of the study. In addition, ADS and DJL set up the Epistasis Project, with the help of the other authors. ADS and DJL decided on the strategy of the Epistasis Project, with the help of CMvdO, DC, KM, PK, RH, MC-B, DRW and EC. ADS, DJL, CMvdO, OK, KM, PK, RH, MC-B, DRW and EC chose the genetic interactions to study. DC and IM produced the hypothesis for this study. KM and OB gave extensive advice on the choice of SNPs to study. DJL made the final selection of polymorphisms. HK, RB, KM, DRW, EC and IM provided DNA for genotyping. DRW gave technical advice throughout. RG and NH were responsible for the genotyping of 6 sample-sets. AA-V was responsible for the Rotterdam genotyping. MC-B and DJL decided on the analytical approach. MC-B and AO advised on statistics. DJL, MGL, MC-B and AO performed the analyses. DJL drafted the manuscript. OC submitted the manuscript and is responsible for correspondence. All authors read the manuscript, studied it critically for its intellectual content and approved the final draft.

Competing interests

The authors declare that they have no competing interests.

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