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Alzheimer's Disease Risk Variant rs2373115 Regulates *GAB2* and *NARS2* Expression in Human Brain Tissues

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Abstract

Genetic association studies have identified significant association between the *GAB2* rs2373115 variant and Alzheimer's disease (AD). However, it is unknown whether rs2373115 affects the regulation of nearby genes. Here, we evaluate the potential effect of rs2373115 on gene expression using multiple eQTL (expression quantitative trait loci) datasets from human brain tissues from the Mayo Clinic brain expression genome-wide association study (eGWAS), the UK Brain Expression Consortium (UKBEC), the Genotype-Tissue Expression (GTEx) project, and the Brain xQTL Serve. Our findings indicate that the rs2373115 C allele is associated with increased *NARS2* expression, and both reduced and increased *GAB2* expression in human tissues. Using a large-scale AD case-control expression dataset, we found increased *GAB2* expression and reduced *NARS2* expression in AD cases compared with controls. We believe that our findings provide important information regarding the rs2373115 variant and expression of nearby genes with respect to AD risk.

Keywords Alzheimer's disease · *GAB2* · Genome-wide association study · eQTLs

Introduction

Alzheimer's disease (AD) is the most common disease of dementia and neurodegeneration in the elderly (Jiang et al. 2017). AD is highly heritable and complex (Bao et al. 2015). In recent years, genome-wide association studies (GWAS) and pathway analysis of GWAS have been widely performed (Bao et al. 2015). Interestingly, these studies have identified several AD risk variants and pathways associated with the potential pathogenesis and genetic mechanisms of AD (Chen et al. 2015; Liu et al. 2017b; Shen et al. 2015; Zhang et al. 2015).

Genetic variants, such as single nucleotide polymorphisms (SNPs), may affect gene expression in disease-relevant tissues (Bao et al. 2015; Liu et al. 2015; Liu et al. 2017a; Liu et al. 2016). SNPs influencing gene expression are an important class of functional variant, and are named expression quantitative trait loci (eQTLs) (Allen et al. 2012; Bao et al. 2015; Liu et al. 2015; Liu et al. 2017a; Liu et al. 2017c). Taking AD risk variants as an example, Allen et al. evaluated the potential cis-association between AD risk variants and gene expression levels of nearby AD risk genes (*ABCA7*, *BIN1*, *CLU*, *MS4A4A*, *MS4A6A*, *PICALM*), and of 13 genes within ± 100 kb of these SNPs (*MS4A7*, *EED*, *SCARA3*, *EPHX2*, *ZYX*, *FAM131B*, *MS4A14*, *CNN2*, *SBNO2*, *GPX4*, *ARID3A*, *C19orf6*, and *WDR18*) (Allen et al. 2012). In 2015, Allen et al. tested 12 AD risk variants (*CR1*, *CD2AP*, *INPP5D*, *MEF2C*, *HLA-DRB-5/HLA-DRB-1*, *NME8*, *ZCWPW1*, *PTK2B*, *CELF1*, *SORL1*, *FERMT2*, *SLC24A4/RIN3*, and *CASS4*) for cis-association with the gene expression levels of 34 genes within ± 100 kb (Allen et al. 2015).

In 2007, GWAS highlighted the involvement of the *GAB2* rs2373115 (C > A) variant in AD risk with $P = 9.0E\text{-}11$, odds ratio (OR) = 4.06 for the C allele, and 95% (CI) 2.81–14.69 in a European population (Reiman et al. 2007). In 2013, a large-

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scale AD GWAS meta-analysis with 17,008 AD cases and 37,154 controls further confirmed the above association with $P = 8.40E-03$ and OR = 1.06 for the C allele in a European population (Lambert et al. 2013). Our recent findings suggest that *GAB2* rs2373115 contributes to increased AD susceptibility only in the European population but not in the East Asian population (Hu et al. 2017b).

It is unknown whether rs2373115 can regulate the expression of nearby genes, such as *GAB2* and *NARS2* (Hu et al. 2017b). Here, we evaluated this potential cis-association using multiple eQTL datasets from human brain tissues from six brain expression GWAS, the UK Brain Expression Consortium (UKBEC), the Genotype-Tissue Expression (GTEx) project, and Brain xQTL Serve. We also analyzed a large-scale gene expression dataset to evaluate the expression of *GAB2* and *NARS2* in AD cases and controls.

Materials and Methods

Mayo eQTL Dataset

The Mayo eQTL dataset includes six brain-expression GWAS eQTL datasets from 773 brain samples of the human cerebellum and temporal cortex (Zou et al. 2012). In summary, these 773 samples consist of 177 non-AD cerebellar samples, 197 non-AD temporal cortex samples, 197 AD cerebellar samples, 202 AD temporal cortex samples, [that is 374 cerebellum (197 AD and 177 non-AD), and 399 temporal cortex (202 AD and 197 non-AD) samples] (Zou et al. 2012). The non-AD samples have brain pathologies including progressive supranuclear palsy, Lewy Body Disease, corticobasal degeneration, frontotemporal lobar degeneration, multiple system atrophy, and vascular dementia (Zou et al. 2012).

Braineac eQTL Dataset

The Braineac eQTL dataset is from the UKBEC (Ramasamy et al. 2014). This dataset includes 10 brain regions from 134 neuropathologically normal individuals of European descent (Ramasamy et al. 2014). The 10 brain regions are cerebellar cortex, frontal cortex, hippocampus, medulla, occipital cortex, putamen, substantia nigra, temporal cortex, thalamus, and intralobular white matter (Ramasamy et al. 2014). The gene expression levels include the exon-specific level and the transcript level (Winsorized mean over exon-specific levels). More detailed information is described in the original study (Ramasamy et al. 2014).

GTEx eQTL Dataset

The GTEx (version 6) includes 53 tissues, 544 donors, and 8555 samples. These 544 donors had several different fatal pathologies including traumatic injury, cerebrovascular disease, heart disease, liver, renal, respiratory, and neurological diseases (GTEx Consortium 2013). Here, we limit our analysis to 10 human brain tissues, each including at least 70 samples. These 10 human brain tissues include anterior cingulate cortex, caudate, cerebellar hemisphere, cerebellum, cortex, frontal cortex BA9, hippocampus, hypothalamus, nucleus accumbens, and putamen (GTEx Consortium 2015).

xQTL Dataset

We selected another large-scale eQTL dataset including 494 human prefrontal cortex samples from Brain xQTL Serve (Ng et al. 2017). About 96% of these 494 samples were diagnosed with neurodegenerative disease. The remaining 4% ($n = 17$) of participants were diagnosed to be the neuropathologically normal individuals (Ng et al. 2017).

eQTL Analysis

In brief, a linear regression analysis was applied to evaluate the potential cis-association between eQTLs and gene expression under an additive model by adjusting for some critical covariates. In the Mayo eQTL dataset, these included APOE ε4 dosage, age at death, sex, PCR plate, RIN, and adjusted RIN squared $(\text{RIN-RINmean})^2$ (Zou et al. 2012). In the Braineac dataset including the brain bank, gender, and batch effects in Partek's Genomics Suite v6.6 (Ramasamy et al. 2014), and GTEx dataset including top 3 genotyping principal components, a set of covariates was identified using the Probabilistic Estimation of Expression Residuals method, genotyping array platform, and sex (GTEx Consortium 2015). In the xQTL dataset, Spearman's rank correlation was used to perform the eQTL analysis by adjusting for several critical covariates, including the effects of RNA integrity score, postmortem interval, sequencing depth, study index, genotyping PCs, age at death, and sex (Ng et al. 2017).

From the Braineac database, we downloaded the gene expression data and the genotype data of generic variants for 1 Mb upstream and 1 Mb downstream of the transcription start site (Ramasamy et al. 2014). We further utilized the R program to evaluate the potential cis-association between rs2373115 and gene expression of nearby genes. Meanwhile, we downloaded the summary results from the Mayo eQTL dataset, or used the online GTEx (version 6) database and xQTL database, to directly evaluate the potential association between rs2373115 and expression of nearby genes. The significance level for eQTL analysis was defined as $P < 0.05$.

Gene Expression Analysis

To further evaluate differential expression of *GAB2* and *NARS2* in AD cases and controls, we selected a large-scale

Table 1 rs2373115 C allele and gene expression in six GWAS datasets

Brain tissues	Samples	Beta	*P* value	Gene	Illumina probe ID
Cerebellum_AD	187	0.121	*3.09E-06**	NARS2	ILMN_1797332
Cerebellum_AD	187	− 0.089	*1.51E-02*	GAB2	ILMN_1728746
Cerebellum_AD	187	− 0.113	*2.84E-02*	GAB2	ILMN_1815758
Cerebellum_AD	187	0.039	1.74E-01	GAB2	ILMN_1665964
Cerebellum_All	361	0.093	*5.15E-07*	NARS2	ILMN_1797332
Cerebellum_All	361	− 0.056	*2.41E-02*	GAB2	ILMN_1728746
Cerebellum_All	361	0.045	5.22E-02	GAB2	ILMN_1665964
Cerebellum_All	361	− 0.065	7.39E-02	GAB2	ILMN_1815758
Cerebellum_Control	174	0.073	*8.73E-03*	NARS2	ILMN_1797332
Cerebellum_Control	174	0.055	1.45E-01	GAB2	ILMN_1665964
Cerebellum_Control	174	− 0.021	5.47E-01	GAB2	ILMN_1728746
Cerebellum_Control	174	− 0.006	9.04E-01	GAB2	ILMN_1815758
Temporal cortex_AD	194	0.07	*4.77E-03*	NARS2	ILMN_1797332
Temporal cortex_AD	194	− 0.205	*6.28E-03*	GAB2	ILMN_1728746
Temporal cortex_AD	194	0.029	4.35E-01	GAB2	ILMN_1665964
Temporal cortex_AD	194	0.022	7.62E-01	GAB2	ILMN_1815758
Temporal cortex_All	377	0.098	*2.59E-07*	NARS2	ILMN_1797332
Temporal cortex_All	377	− 0.241	*5.09E-06*	GAB2	ILMN_1728746
Temporal cortex_All	377	− 0.046	4.03E-01	GAB2	ILMN_1815758
Temporal cortex_All	377	0.004	8.72E-01	GAB2	ILMN_1665964
Temporal cortex_Control	183	0.131	*9.13E-06*	NARS2	ILMN_1797332
Temporal cortex_Control	183	− 0.264	*3.65E-04*	GAB2	ILMN_1728746
Temporal Cortex_Control	183	− 0.11	1.75E-01	GAB2	ILMN_1815758
Temporal cortex_Control	183	− 0.015	7.34E-01	GAB2	ILMN_1665964

*Significant associations ($P < 0.05$) are in italic type

Beta is the regression coefficient, based on the rs2373115 C allele using an additive model. rs2373115 position, chromosome 11, 78091150 bp (hg19); Beta > 0 and Beta < 0 mean that this allele is associated with increased and reduced gene expression, respectively

human brain gene expression dataset including 176 late-onset AD cases and 187 controls from the NCBI GEO database (GSE15222) (Webster et al. 2009). Here, we used the online web tool GEO2R (Clough and Barrett 2016) to identify whether *GAB2* and *NARS2* are differentially expressed in AD and control groups. The significance level for differential expression was defined as $P < 0.05$. Meanwhile, we extracted the *GAB2* and *NARS2* expression data from these 363 samples. We then performed Pearson's product-moment correlation analysis to investigate the potential association between the expression levels of *GAB2* and *NARS2* using R program. The significance level for correlation analysis was defined as $P < 0.05$.

Results

eQTL Analysis of the Mayo Dataset

In the human cerebellum and temporal cortex tissues, the rs2373115 C allele was significantly associated with *NARS2* expression ($P < 0.05$) in all six brain expression GWAS datasets. Interestingly, only the C allele was associated with increased *NARS2* expression (Beta > 0), as described in Table 1. The C allele was also significantly associated with *GAB2* expression ($P < 0.05$) in five brain expression GWAS datasets, but not in the cerebellar samples of the non-AD subjects. In contrast with *NARS2*, the rs2373115 C allele was only significantly associated with reduced *GAB2* expression (Beta < 0), as described in Table 1.

eQTL Analysis of the Braineac Dataset

In the Braineac dataset, the rs2373115 C allele was significantly associated with *NARS2* expression ($P < 0.05$) and only with increased *NARS2* expression (Beta > 0) in five of the 10 brain tissues, including the cerebellar cortex, frontal cortex, occipital cortex, putamen, and temporal cortex, as described in Table 2. However, the C allele was not significantly associated with *GAB2* expression in any of the 10 tissues examined ($P > 0.05$), as described in Table 2.

Table 2 rs2373115 C allele and gene expression in the Braineac dataset

Brain tissues	Samples	Beta	*P* value	Gene	Experiment ID
Cerebellar cortex	134	0.1433	*7.85E-03**	NARS2	t3383322
Frontal cortex	134	0.1646	*1.06E-02*	NARS2	t3383322
Hippocampus	134	0.1181	5.86E-02	NARS2	t3383322
Medulla	134	0.1163	5.72E-02	NARS2	t3383322
Occipital cortex	134	0.1673	*1.96E-02*	NARS2	t3383322
Putamen	134	0.1871	*5.16E-03*	NARS2	t3383322
Substantia nigra	134	0.1219	1.43E-01	NARS2	t3383322
Temporal cortex	134	0.2104	*7.85E-04*	NARS2	t3383322
Thalamus	134	0.0418	5.00E-01	NARS2	t3383322
Intralobular white matter	134	0.0815	1.56E-01	NARS2	t3383322
Cerebellar cortex	134	0.0006	9.95E-01	GAB2	t3383227
Frontal cortex	134	0.0758	3.57E-01	GAB2	t3383227
Hippocampus	134	0.0134	8.61E-01	GAB2	t3383227
Medulla	134	0.051	4.06E-01	GAB2	t3383227
Occipital cortex	134	0.0436	5.68E-01	GAB2	t3383227
Putamen	134	0.0496	5.99E-01	GAB2	t3383227
Substantia nigra	134	−0.1174	1.14E-01	GAB2	t3383227
Temporal cortex	134	−0.0052	9.49E-01	GAB2	t3383227
Thalamus	134	0.0151	8.33E-01	GAB2	t3383227
Intralobular white matter	134	0.0161	8.24E-01	GAB2	t3383227

*Significant associations ($P < 0.05$) are in italic type

Beta is the regression coefficient, based on the rs2373115 C allele using an additive model. rs2373115 position, chromosome 11, 78091150 bp (hg19); Beta > 0 and Beta < 0 mean that this allele is associated with increased and reduced gene expression, respectively

eQTL Analysis of the GTEx Dataset

In the GTEx dataset, the rs2373115 C allele was significantly associated with increased NARS2 expression ($P < 0.05$) (Beta > 0) in all 10 brain tissues examined, as described in Table 3. Compared with *NARS2*, the C allele was only significantly associated with reduced *GAB2* expression in the frontal cortex BA9 (Beta = − 0.277, $P = 4.00E-02$), as described in Table 3.

eQTL Analysis of the xQTL Dataset

In the xQTL dataset, the rs2373115 variant C allele was only significantly associated with increased *GAB2* and *NARS2* expression in human prefrontal cortex, as described in Table 4. Compared with the other eQTL datasets above, the association between the rs2373115 C allele and the direction of *GAB2* expression may be different in different tissues.

Gene Expression Analysis

The gene expression dataset (GSE15222) included three probes for *GAB2* (GI_18105040-I, GI_18105041-A, and GI_18105041-I) and one probe for *NARS2* (GI_39725682-S). We identified significantly increased *GAB2* expression in AD cases compared with controls for GI_18105041-A (nominal $P = 1.08E-03$, fold change = 1.19), but not for GI_18105040-I (nominal $P = 0.165$, fold change = 1.18), or GI_18105041-I (nominal $P = 0.935$, fold change = 1.01). We also identified significantly reduced *NARS2* expression in AD cases compared with controls (nominal $P = 2.08E-02$, fold change = 0.86).

We extracted the *GAB2* and *NARS2* expression data from 363 samples. Pearson's product-moment correlation analysis showed significant correlation of *NARS2* with *GAB2* for GI_18105040-I (correlation estimate = − 0.19, and $P = 0.0002286$), and GI_18105041-A (correlation estimate = − 0.47, and $P < 2.2E-16$), but showed no significant correlation of *NARS2* with *GAB2* for GI_18105041-I (correlation estimate = − 0.07, and $P = 0.177$).

Discussion

GAB2 is a member of the GAB gene family, whose members participate in multiple signaling pathways (Pan et al. 2010; Schjeide et al. 2009). In 2007, Reiman et al. found that *GAB2* overexpression in pathologically vulnerable neurons could modify AD risk in APOE ε4 carriers and influence AD neuropathology (Reiman et al. 2007). In 2010, Pan et al. reviewed the role of GAB2 protein in the pathogenesis of AD

Table 3 rs2373115 C allele and gene expression in the GTEx dataset

Brain tissues	Samples	Beta	*P* value	Gene	Experiment ID
Anterior_cingulate_cortex_BA24	72	0.439	*4.13E-04**	NARS2	ENSG00000137513.5
Caudate_basal_ganglia	100	0.315	*1.75E-02*	NARS2	ENSG00000137513.5
Cerebellar_hemisphere	89	0.56	*2.85E-06*	NARS2	ENSG00000137513.5
Cerebellum	103	0.309	*1.04E-02*	NARS2	ENSG00000137513.5
Cortex	96	0.512	*6.02E-04*	NARS2	ENSG00000137513.5
Frontal_cortex_BA9	92	0.487	*1.22E-03*	NARS2	ENSG00000137513.5
Hippocampus	81	0.289	*2.93E-02*	NARS2	ENSG00000137513.5
Hypothalamus	81	0.39	*1.13E-02*	NARS2	ENSG00000137513.5
Nucleus_accumbens_basal_ganglia	93	0.318	*4.75E-02*	NARS2	ENSG00000137513.5
Putamen_basal_ganglia	82	0.605	*3.37E-04*	NARS2	ENSG00000137513.5
Anterior_cingulate_cortex_BA24	72	0.178	1.06E-01	GAB2	ENSG00000033327.8
Caudate_basal_ganglia	100	−0.007	9.18E-01	GAB2	ENSG00000033327.8
Cerebellar_hemisphere	89	0.168	2.06E-01	GAB2	ENSG00000033327.8
Cerebellum	103	0.182	6.93E-02	GAB2	ENSG00000033327.8
Cortex	96	0.038	6.81E-01	GAB2	ENSG00000033327.8
Frontal_cortex_BA9	92	−0.277	*4.00E-02*	GAB2	ENSG00000033327.8
Hippocampus	81	0.047	6.31E-01	GAB2	ENSG00000033327.8
Hypothalamus	81	0.023	7.80E-01	GAB2	ENSG00000033327.8
Nucleus_accumbens_basal_ganglia	93	−0.005	9.63E-01	GAB2	ENSG00000033327.8
Putamen_basal_ganglia	82	0.121	9.14E-02	GAB2	ENSG00000033327.8

*Significant associations ($P < 0.05$) are in italic type

Beta is the regression coefficient, based on the rs2373115 C allele using an additive model. rs2373115 position, chromosome 11, 78091150 bp (hg19); Beta > 0 and Beta < 0 mean that this allele is associated with increased and reduced gene expression, respectively

(Pan et al. 2010). In 2012, Hibar et al. analyzed 755 young-adult twins, and identified a significant association between *GAB2* and morphological brain differences (Hibar et al. 2012). In 2013, Zou et al. measured *GAB2* mRNA levels in 249 brains, and found that increased *GAB2* mRNA levels were significantly associated with decreased AD pathology, including decreased neurofibrillary tangle and senile plaque counts (Zou et al. 2013).

Recent genetic association studies have identified the involvement of the *GAB2* rs2373115 variant in AD (Belbin et al. 2011; Ikram et al. 2009; Naj et al. 2011; Reiman et al. 2007). Here, we evaluated this potential cis-association between rs2373115 and expression of nearby genes using multiple eQTL datasets from different diagnostic groups and brain tissue regions.

Using the six brain expression GWAS datasets from AD and non-AD subjects with different brain pathologies, we identified that the rs2373115 C allele was associated with increased *NARS2* expression and reduced *GAB2* expression in the human cerebellum and temporal cortex. Using the Braineac dataset of 134 neuropathologically normal individuals, we found that the rs2373115 C allele was associated with increased *NARS2* expression, but not with reduced *GAB2* expression. In the GTEx dataset, the rs2373115 C allele was significantly associated with increased *NARS2* expression in all 10 brain tissues examined. However, the rs2373115 C allele was only significantly associated with reduced *GAB2* expression in one brain tissue. In the xQTL dataset, we further found that the rs2373115 C allele was only associated with increased *GAB2* and *NARS2* expression in the prefrontal cortex.

These findings prompt us to conclude that the rs2373115 C allele may have more widespread effects on the expression of *NARS2* compared with *GAB2*; the rs2373115 C allele may need tissue- and disease-specific factors to exert its influences on *GAB2* expression. The rs2373115 C allele may influence *NARS2* expression in numerous human brain regions and in different diagnostic groups including AD cases (Zou et al. 2012), non-AD subjects with different brain pathologies (Zou et al. 2012), neuropathologically normal individuals (Ramasamy et al. 2014), and subjects with various fatal pathologies (GTEx Consortium 2015). Our findings also suggest that the rs2373115 C allele may influence *GAB2* expression in specific human brain regions. This association with expression may be tissue dependent and only be observed in certain brain regions, such as the cerebellum, temporal cortex, and prefrontal cortex (Ng et al. 2017; Zou et al. 2012). However this association may be brain disease specific as it is reported in AD cases (Zou et al. 2012), and non-AD subjects with different brain pathologies (Ng et al. 2017; Zou et al. 2012), but not in neuropathologically normal individuals (Ramasamy

Table 4 rs2373115 C allele and gene expression in the xQTL dataset

Brain tissues	Samples	Spearman's rank correlation	*P* value	Gene
Prefrontal cortex	494	0.22	*7.65E-07**	GAB2
Prefrontal cortex	494	0.2	*7.82E-06**	NARS2
Prefrontal cortex	494	0.088	0.050	CLNS1A
Prefrontal cortex	494	0.075	0.095	AQP11
Prefrontal cortex	494	−0.065	0.146	ODZ4
Prefrontal cortex	494	0.06	0.187	USP35
Prefrontal cortex	494	−0.048	0.283	NDUFC2
Prefrontal cortex	494	0.047	0.300	RSF1
Prefrontal cortex	494	0.034	0.450	KCTD21
Prefrontal cortex	494	−0.029	0.513	ALG8
Prefrontal cortex	494	−0.018	0.684	INTS4
Prefrontal cortex	494	−0.018	0.688	PAK1
Prefrontal cortex	494	0.007	0.885	C11orf67

*Significant associations ($P < 0.05$) are in italic type

Spearman's rank correlation is the regression coefficient, based on the rs2373115 C allele using an additive model. rs2373115 position, chromosome 11, 78091150 bp (hg19); Spearman's rank correlation > 0 and Spearman's rank correlation < 0 mean that this allele is associated with increased and reduced gene expression, respectively

et al. 2014), or subjects with various fatal pathologies (GTEx Consortium 2015).

Using the large-scale AD case-control expression dataset, we found increased *GAB2* expression and reduced *NARS2* expression in AD cases compared with controls. We believe that our findings provide important information regarding the rs2373115 variant and expression of nearby genes with respect to AD risk.

Despite these interesting results, we recognize some limitations. In this study, we aimed to evaluate the potential cis-association, but not the causal association between the rs2373115 C allele and *NARS2*/*GAB2* expression. Our findings should be considered as exploratory. More evidence is needed to confirm a causal relationship; eQTLs analysis alone is not sufficient. There is also a tendency to infer causality when other causal interpretations are possible, and empirical studies are need to demonstrate true causality. In our future work, we will further evaluate whether the association between the rs2373115 C allele and *NARS2*/*GAB2* expression is a causal association using a number of methods and technologies, including analysis of long-range chromatin interactions using CHi-C, genotype-specific 3C, cell-type and genotype-specific eQTL analysis, and chromatin immunoprecipitation (ChIP) (McGovern et al. 2016). Some covariates, including sex, age, and ApoE status may significantly influence gene expression and eQTL analysis (Hu et al. 2017a; Liu et al. 2015; Liu et al. 2017a; Liu et al. 2016; Zou et al. 2012) and it is necessary to perform a stratified analysis. However, all these covariates and clinical phenotype features are not publicly available. When we obtain these covariates and clinical phenotype features, we will further evaluate our findings.

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Compliance with Ethical Standards

Competing Interest The authors declare that they have no competing interests.

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