Sleep Apnoea and Early Antecedents of Adult Disease

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ABSTRACT

Obstructive sleep apnoea (OSA) is a highly prevalent disorder across the life spectrum including early childhood and pregnancy. Occurrence of sleep perturbations and intermittent hypoxia that constitute major hallmarks of OSA during these favourable developmental plasticity windows may facilitate emergence of incremental risks for a variety of ageing-related disorders via a multitude of epigenetic mechanisms. In addition, OSA during late adolescence and adulthood is not immune to epigenetic changes, the latter potentially playing a role in the reversibility of OSA-associated morbidities upon implementation of therapy. Furthermore, unique epigenetic signatures may provide powerful biomarkers for precision-based medicine approaches in the framework of OSA. Taken together, the conceptual umbrellas assigning major roles to epigenetics in the context of OSA-associated phenotypic expression and longitudinal disease risk trajectories is not a farfetched idea any longer. Early adoption of these biologically relevant principles and their implementation to the upcoming future clinical trials appears inevitable if progress is to occur.

Key words: Epigenetics. Intermittent hypoxia. Microbiome. Sleep apnoea. Sleep fragmentation.

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INTRODUCTION

A large and ever-expanding body of evidence indicates that sleep disordered breathing (SDB) is a substantial and prevalent health problem in both the adult and paediatric populations. Indeed, a conservative estimate would suggest that around 5% of children and up to 15-35% of adults in the general population suffer from the most common form of SDB. Obstructive sleep apnoea (OSA) is characterised by recurrent episodes of upper airway obstruction or partial obstruction during sleep that result in intermittent hypoxia (IH) and hypercapnia, sleep disruption, and increased intrathoracic pressure swings due to respiratory effort aimed at opening the collapsed airway. The ever-expanding interest and awareness to OSA has been predicated on the epidemiological and experimental evidence linking OSA with a large repertoire of end-organ morbidities which ultimately result in increased all-cause mortality1,2.

In this context, a substantial body of evidence has suggested that OSA is independently associated with neurocognitive, cardiovascular and metabolic morbidities3-5, and even with increased cancer prevalence and adverse outcomes6-10. However, in a recent review of the evidence focused on the potential value of screening for OSA, ambivalence was expressed as to whether treatment of OSA will reverse its associated morbidities and was found to only significantly improve excessive daytime sleepiness and quality of life11. Furthermore, although hotly debated and contested, a large multicentre study, the Sleep Apnea Cardiovascular Endpoints (SAVE) trial, found no evidence to support the use of continuous positive airway pressure (CPAP) therapy in the secondary prevention of cardiovascular morbidity and mortality in patients with OSA12,13.

These overall findings have suggested that the presence of long-standing disease may adversely affect the reversibility of its consequences, and preliminary experimental evidence in murine models appears to support such assumption and point to the possibility that the presence of some or all of the characteristic perturbations that constitute OSA may foster selective changes in the epigenome, some of which may become irreversible14,15.

Here, I will briefly discuss the current understanding of epigenetics in the context of OSA and review the evidence on how this disease can impose a variety of epigenetic modifications which conditional on their occurrence at various stages of life can then promote risk of long-term morbidities or attenuate the reversibility of such morbidities. I should however emphasise that individuals suffering from OSA at any age are exposed to their unique environmental conditions and evolve in their very personal lifestyle, both of which can also impose major modifications to the cogwheel of OSA-induced morbidity, in addition to the contributions attributable to genetic background and epigenetic modifications discussed below (Fig. 1).

EPIGENETICS AND DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE

Since the original proposal by Barker on the early life origins of human disease16, and the conceptual framework leading to the current eponym of DOHAD (Developmental Origins of
Health And Disease), a large body of literature has provided compelling evidence to the validity of this theoretical construct, and its implications in many of the diseases affecting our society. Indeed, Barker and Osmond found high rates of death due to coronary heart disease in areas with elevated neonatal mortality in England and Wales and put forth the proposal that intrauterine deprivation was an important mediator of such mortality. Numerous studies have subsequently documented associations between low birth weight and increased incidence of heart disease, hypertension, and type 2 diabetes, as well as relevant markers such as insulin resistance and dyslipidaemias. Extrapolation of this early exposure linkage to other diseases such as cancer, neurodegenerative disorders and overall accelerated ageing has gained substantial traction in recent years, further attesting to the importance of internal and external environmental cues to the modulation of homeostatic processes, including those involved in tissue repair and regeneration.

**DEVELOPMENTAL PLASTICITY AND EPIGENETIC PROCESSES**

Developmental plasticity can be defined as the ability of one genotype to produce a range...
of phenotypes in response to environmental conditions. Such plasticity can occur at the level of individual cells, an organ, or the whole organism. As the embryo develops, lineage restriction events reduce cellular pluripotency such that cellular plasticity declines with developmental age. Epigenetic processes represent a major regulatory mechanism by which the environment modulates gene transcription during development and may explain the decrease of cellular plasticity by the establishment of cell-specific epigenetic profiles during development. Moreover, epigenetic processes are responsible for transgenerational inheritance in several complex traits. Epigenetics studies focus on three related molecular mechanisms for genome regulation: deoxyribonucleic acid (DNA) modifications, histone modifications and non-coding ribonucleic acids (ncRNAs). DNA modification is the covalent modification of a nucleotide in the DNA sequence. The most studied DNA modifications are the addition of a methyl or hydroxymethyl group at the fifth position of the cytosine nucleotide (C), termed as 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC), respectively. In the mammalian genome, these additions occur almost exclusively in the context of a cytosine-guanine (CG) dinucleotide and may regulate the expression of the cognate genes. The second epigenetic mechanism in genome regulation is determined by the organization of the histones in the nucleosomes. Histone modifications include methylation, acetylation, phosphorylation, ubiquitination, sumoylation, citrullination, and adenosine diphosphate ribose (ADP)-ribosylation. Either working as individual marks or in a combinatorial pattern, histone modifications are major regulators of gene expression, and can act as activating or repressing marks. The third layer of epigenetic regulation is given by ncRNAs. This category includes several types of RNA molecules which are not coding for proteins, but may have a function on genome regulation, such as micro RNAs (miRNA), small interfering RNAs (siRNA) and long non-coding RNAs (lncRNA). At defined developmental stages, DNA and chromatin are modified leading to variations in gene regulation that will result in the establishment of specific cell lineages (cell differentiation). Environmental factors have an impact on how faithfully patterns of epigenetic modifications are maintained throughout adult life. Furthermore, epigenetic programming can be reversed by environmental interventions in early or adult life, such as treatment with epigenetic drugs, nutrition or maternal care. In this regard, it has been recently reported for example that activities such as exercise induce DNA methylation changes in human skeletal muscle and human adipose tissue, potentially affecting the regulation of organismal metabolism.

In the context of DOHAD, the “thrifty phenotype” hypothesis proposes that poor nutrition in early life produces permanent changes in glucose-insulin metabolism and results in poor foetal and infant growth and subsequent development of type 2 diabetes and metabolic syndrome. In the original report by Barker and Osmond, 407 men born in Hertfordshire (1920-1930), and 266 men and women born in Preston (1935-1943) in the United Kingdom, whose weights and size had been recorded in detail at birth and at one year of age were analysed. The prevalence of the “thrifty phenotype” syndrome correlated inversely with birth weight. This observation indicates that altered gestational conditions leading to “small for gestational age” (SGA)
children might have induced phenotypic changes, which are then manifested as increased cardiometabolic risk in late adulthood. These earlier observations suggested that early environmental exposures, such as gestational hyperglycaemia, maternal obesity and overfeeding during pregnancy, epigenetically alter the programming of genes resulting in a long-term imprint on gene expression that lasts into adulthood. There is now evidence of a sizeable contribution of early-life influences such as maternal high fat diet or malnutrition on the risk of becoming an obese adolescent or adult and of developing obesity-associated diseases in adulthood. Indeed, the rapidly rising incidence of childhood obesity could reflect the same processes, whereby developmental factors will affect adult disease risk via several pathways, all of which depend on developmental plasticity. These long-term effects are now believed to be mediated by epigenetic changes and evidence supportive of such assumptions is emerging\(^{32,47}\).

**OBSTRUCTIVE SLEEP APNOEA DURING PREGNANCY AND CONSEQUENCES TO THE OFFSPRING**

Based on the aforementioned considerations, one of the potential windows of vulnerability linking OSA and epigenetic susceptibility determinants of later onset of disease would be the occurrence of OSA during pregnancy. To this effect, we should be aware that two-thirds of women in the United States are currently overweight or obese at the time of conception, and that pre-conception obesity is fraught with higher risk for the gestation itself and for the foetal outcomes\(^{48-52}\). Furthermore, paternal obesity is not without risk either\(^{53}\). In addition, early in gestation - first and early second trimester - maternal insulin sensitivity can actually increase modestly\(^{54}\), leading to elevated maternal adipose tissue lipid storage.

Pregnancy, and particularly late gestation, has been associated with a relatively high prevalence of altered sleep patterns including restricted sleep duration, fragmented sleep, snoring, and especially OSA\(^{55-67}\). Experimental evidence in murine models has conclusively demonstrated that OSA can induce changes in insulin sensitivity and promote obesogenic behaviours as well as hypertension, i.e., correlates of pre-eclampsia\(^{68-71}\). Similarly, preliminary findings in pregnant women suggest that disrupted sleep, specifically as a result of snoring or OSA is associated with increased risk for insulin resistance and pre-eclampsia\(^{75-81}\). Perturbations in sleep duration during pregnancy are also suggested to be accompanied by altered function in the mother\(^{82-84}\) and also affect developmental trajectories in the offspring\(^{85}\).

Sleep fragmentation (SF) and IH are fundamental characteristics of OSA. Both paradigms can be induced individually or in combination in rodent models to study the effects of OSA\(^{86}\). Offspring of rats exposed to IH showed lower birth weight and an increased propensity for high body weight later in life when compared with offspring of pregnant rats exposed to normal oxygen levels – room air\(^{87}\). Several recent studies indicate that snoring mothers are more likely to have SGA infants and exhibit higher risk for perinatal complications\(^{88-92}\). Furthermore, and as a validity preamble to the putative distal yet adverse role of OSA on the overall well-being of the offspring, we have shown that implementation
of the hallmark characteristics of OSA in experimental mice during late gestation promotes the risk of their offspring developing significant metabolic dysfunction when they reach adulthood. Indeed, exposure of pregnant mice to SF during late gestation is accompanied by increased risk of metabolic dysfunction in offspring manifesting as increased body weight accrual, dyslipidaemia and insulin resistance. In addition, systematic exploration of epigenetic changes in the offspring revealed adiponectin gene (Adipoq) epigenetic alterations in adipocytes from visceral white adipose tissue as well as forkhead box protein 01 (Foxo1) epigenetic changes in liver in a sex-dimorphic pattern of expression. Genome-wide exploration of the offspring revealed 2148 differentially methylated regions (DMR)-associated genes with known functions that are mechanistically relevant to obesity and metabolic syndrome, such as cocaine- and amphetamine-regulated transcript prepopeptide (Cartpt), RAC-beta serine/threonine-protein kinase (Akt2), apolipoprotein E (ApoE), insulin receptor 1 (Insr1), and so on, such that the overrepresented pathways and gene networks were primarily related to metabolic regulation and inflammatory responses. In addition, involvement of the unfolded protein response has been identified, whereby activation of the cellular integrated stress response appears to be critical during gestation to induce the affected phenotype in the offspring, via alterations in circulating exosomal miRNAs. More recently, we also explored the consequences of experimental IH during late gestation and mimicking OSA desaturation patterns on the offspring. Similar to the aforementioned study, body weight, food intake, adiposity index, fasting insulin, triglycerides and cholesterol levels were all significantly higher in gestationally-exposed male offspring at 24 weeks of age (corresponding to mid adulthood in human) but not in exposed female offspring. Late gestational IH also altered metabolic expenditure and locomotor activities in male offspring, and increased number of pro-inflammatory macrophages in their visceral white adipose tissues. Assessment of methylation changes in the genomic DNA of adipocytes from visceral fat revealed 1520 DMRs associated with 693 genes, corresponding to molecular pathways related to metabolic regulation and inflammation. Thus, both gestational SF and IH induce metabolic dysfunction as reflected by increased body weight and adiposity index in adult male offspring that is paralleled by epigenetic alterations and inflammation in visceral fat, suggesting that perturbations to foetal environment by OSA during pregnancy can have long-term detrimental effects on the foetus, and lead to persistent metabolic dysfunction in adulthood via epigenetic changes (Fig. 2). Prospective studies aimed at identifying unique epigenetic risk signatures in offspring of mothers suffering from OSA during pregnancy would be highly desirable and will likely take place once the initial large scale epidemiological studies on OSA in pregnancy are completed.

In addition to these considerations, we should also contemplate the possibility that unique conditions during gestation facilitating the emergence of premature birth may in turn impose an undue risk of developing OSA later in life. This specific perspective is much less developed in the current published literature, and the only evidence to this effect is that children who were born prematurely...
are at a greater risk of suffering from OSA as they grow\textsuperscript{101}. To date, it remains unclear whether there is a causal relation between the determinants of pre-term birth and the long-term development of the various contributing mechanisms to the occurrence of upper airway dysfunction. However, one could easily contemplate that perturbations during critical gestational periods that would induce premature delivery may also differentially and epigenetically modify unique gene subsets involved for example in craniofacial skeletal growth or aspects of respiratory control that are relevant to sleep-disordered breathing. These hypothetical scenarios merit future studies, particularly considering that identification of a set of risk biomarkers for future development of OSA may enable precision medicine and risk prevention approaches.

**STUDIES ON OBSTRUCTIVE SLEEP APNOEA IN CHILDREN AND EPIGENETIC MODIFICATIONS**

We have thus far explored the potential impact of gestational OSA on offspring. However, the epigenetic window of susceptibility...
is not closed at birth, and rather extends in progressively declining fashion throughout life. Thus, the possibility that the presence of OSA during infancy and childhood may lead to epigenetic changes is not far-fetched. The cumulative evidence in this context is more limited however and will be reviewed here.

Initial studies in our laboratory indicated that early exposures to IH, mimicking apnoea of prematurity, were accompanied by evidence of delayed brain and spinal cord myelination that persisted for extensive periods after cessation of the exposures\textsuperscript{102}. Similarly, exposures of young rats to IH during a critical developmental period were accompanied by long-term alterations in frontal cortical dopaminergic pathways that were sexually dimorphic as far as the persistence of neurobehavioural deficits\textsuperscript{103}. Incidentally, since our initial observations of persistent respiratory changes in the context of IH exposures during early post-natal life\textsuperscript{87}, subsequent work identified age-related declines in respiratory plasticity following exposures to IH that may persist into adulthood\textsuperscript{104-107}.

In more recent studies, Nanduri et al.\textsuperscript{108} identified epigenetic changes in the carotid body as one of the potential mechanisms associated with deregulation of respiratory control following exposures to IH. Under the overarching hypothesis raised by our work above that the IH of apnoea of prematurity predisposes to autonomic dysfunction in adulthood, these investigators showed that adult rats that were exposed to IH as neonates exhibited exaggerated responses to hypoxia by the carotid body and adrenal chromaffin cells, which regulate cardio-respiratory function, and that such persistent alterations resulted in irregular breathing with apnoea and hypertension. Nanduri et al.\textsuperscript{108} further demonstrated that the reduced expression of the superoxide dismutase 2 (SOD2) gene, which encodes the antioxidant enzyme superoxide dismutase 2, was associated with increased methylation of a single C-phosphate-G (CpG) dinucleotide in close proximity to the transcription start site of the gene, and that administration of decitabine, an inhibitor of DNA methylation, during neonatal IH exposures prevented the expression of the phenotype in adulthood. Of note, when these exposures were implemented in adult rats, the reversal of the phenotype occurred only after short exposures (10 days) but not prolonged exposures (30 days), and evidence for increased DNA methylation of genes encoding anti-oxidant enzymes was also reported\textsuperscript{109}.

It is now well established that premature birth, and possibly the presence of apnoea of prematurity, are accompanied by an increased risk of cardiovascular and metabolic disease during adulthood\textsuperscript{110-112}. To further examine the viability of the epigenetic hypothesis, we exposed neonatal mice to IH, and then allowed them to recover in normoxia till they reached adulthood\textsuperscript{113}. IH-exposed mice manifested evidence of endothelial dysfunction, as shown by reduced reperfusion indices after tail vessel occlusion indicating abnormal flow mediated vasodilation. Furthermore, the IH-exposed mice also exhibited impaired vasodilatory responses to acetylcholine, and endothelial cells that were harvested from their mesenteric arteries expressed higher levels of angiotensin-converting enzyme (ACE) and reactive oxygen species, along with elevated plasma...
angiotensin-II (AGT) levels. Furthermore, increased DNA methylation patterns of the ACE1 and the AGT genes were apparent in the endothelial cells of the IH-exposed mice\textsuperscript{113}.

To the best of my knowledge, only two studies examining evidence for epigenetic modifications have been conducted thus far in children with OSA. In the initial study, Kim et al\textsuperscript{114} showed that forkhead box P3 (FOXP3) gene, which critically regulates the conversion and function of T regulatory lymphocytes, is more likely to display increased methylation in the promoter region among children with OSA who exhibit increased systemic inflammatory responses, the latter being associated with increased risk for end-organ morbidity. Similarly, the heterogeneously increased risk for cardiovascular morbidity in children with OSA was examined using endothelial post-occlusive hyperaemic responses, which are dependent on the expression and function of endothelial nitric oxide synthase gene (NOS3). Since expression of the NOS3 gene is regulated by epigenetic mechanism, we explored whether children with OSA and abnormal endothelial function showed evidence of increased methylation of NOS3 compared to children with OSA but preserved endothelial function\textsuperscript{115}. Among the 36 children with OSA with endothelial dysfunction ($n = 11$) and preserved endothelial function ($n = 25$), as well as 25 controls without OSA, pyrosequencing of the proximal promoter region of the eNOS gene revealed significant differences in methylation among the OSA children with abnormal endothelial function in a CpG site located at position -171 (relative to transcription start site), approximating important transcriptional elements, suggesting that the reduced expression of NOS3 in these children may be due to the increased methylation of the NOS3 gene. It will be important to evaluate the potential relationships between reversibility of these epigenetic changes and their concordance with reversal of end-organ morbidity following treatment of OSA. Furthermore, longitudinal tracking of unique epigenetic signatures from childhood into adulthood among OSA patients may permit identification of at-risk individuals for premature development of cardiometabolic or neurodegenerative diseases.

**STUDIES ON OBSTRUCTIVE SLEEP APNOEIA IN ADULTS AND EPIGENETIC MODIFICATIONS**

There is only very scarce evidence regarding the occurrence of epigenetic changes in the context of OSA in adults. The initial study followed on our characterization in murine models of the effect of IH simulating OSA on malignant solid tumours\textsuperscript{116}. In this context, we wished to identify circulating tumour cell free DNA (ctDNA) in mice with a standardised solid tumour who were exposed to IH and corresponding controls (CO) and assess whether IH elicited epigenetic changes in tumoural DNA\textsuperscript{117}. Significant associations between plasma ctDNA concentrations and tumour size, weight and invasiveness emerged. More interestingly, using a methylation microarray-based approach, we identified 2094 gene regions showing significant differential ctDNA modifications that reflected unique sets of molecular pathways that are deregulated in cancer progression and transcription start sites in highly variable regions in chromosomes 7, 13, 14 and X that may reflect hotspots for DNA deletions. As such, exposures to IH increase
the shedding of ctDNA into circulation, which carries epigenetic modifications that may characterise cell populations within the tumour and provide opportunities for precision therapeutic approaches. Indeed, a subset of differentially regulated microRNAs within circulating exosomes in OSA murine models provide corroborating evidence that epigenetic mechanisms may play a substantial role in the connection between OSA and cancer\textsuperscript{118,119}.

In a recent study of 44 obese patients with OSA aiming to analyse the influence of resting oxygen consumption on global and gene DNA methylation as well as protein secretion of inflammatory markers in blood cells, López-Pascual et al.\textsuperscript{120} found interleukin 6 (IL-6) gene CpG islands showing evidence of hypomethylation, concordant with serum IL-6 levels being higher in those OSA patients with lower resting energy expenditure. It is therefore possible that epigenetic modifications in inflammatory genes may contribute to the systemic inflammation that is frequently, albeit not universally present among OSA patients.

Finally, we reported on the effects of positive airway pressure therapy on genome-wide epigenetic methylation patterns in monocytes of patients with obesity hypoventilation syndrome. In this study, we found 1847 regions showing significant differential DNA methylation, and bioinformatics analysis of biochemical pathways and gene networks revealed that the differentially methylated genes underlie components of immune responses, and more particularly those targeted by peroxisome proliferation-activated receptors (PPARs), a cluster of genes that play major roles in cardiometabolic and neurodegenerative diseases\textsuperscript{121-125}.

These findings are intimately aligned with the reversibility concept mentioned above. Indeed, in studies involving mice exposed to IH for extended periods of time, we found substantial differential methylation patterns in macrophages within the aorta wall which extensively involved PPARs, and cessation of IH exposures did not appear to reverse the methylation changes or reduce the pro-atherogenic activity of the macrophages within the aortic wall\textsuperscript{15}.

An ongoing clinical trial aimed to resolve some of these issues will hopefully provide some answers to the reversibility questions regarding the morbidity of OSA\textsuperscript{126}. In the interim, a specific miRNA signature has been identified as a reliable predictor of CPAP therapeutic response in patients with OSA and resistant hypertension, opening the doors of personalised approaches in this disease\textsuperscript{127}.

**Evolving Concepts on Microbiome and Obstructive Sleep Apnoea-Induced Morbidity**

The microbiome is a vast, complex and highly regulated microbial community that cohabitates with every other living organism. It is inordinately susceptible and reactive to a large repertoire of both intrinsic and extrinsic changes in the host, and concurrently plays major roles in many of the host functional systems\textsuperscript{128-130}. Recent studies have indicated that altered gut microbiome in the mother during gestation can impose profound changes in the offspring phenotype by altering the
offspring microbiome, and that such changes can occur both via placenta or during the early transitional periods after birth at the time of the constitutional evolution of the microbiome in the offspring\textsuperscript{131-134}. Congruent with these observations, experimentally-induced modifications of the maternal microbiome may result in a variety of dysfunctional phenotypes in the offspring\textsuperscript{135-140}, and even elicit specific epigenetic changes\textsuperscript{141}. Although there is no published evidence as to whether the presence of OSA modified the maternal microbiome, it would be unlikely that such changes would not occur, particularly considering the fact that gut microbiota are extensively altered by the presence of IH, SF, or sleep patterns in mice\textsuperscript{142-144}. Furthermore, OSA-associated changes in gut microbiota have been inferred in children with OSA\textsuperscript{145}, appear to be associated with both cardiovascular and metabolic consequences\textsuperscript{144, 146, 147}, and are not readily reversible\textsuperscript{148}. Accordingly, it will be important to explore how OSA during pregnancy affects the maternal, placental, and offspring microbiota and their contributions to gestational and offspring outcomes. In addition, the potential modification of long-term risks imposed by OSA may be modifiable by targeted interventions of the microbiome.
CONCLUSIONS

Experimental murine models mimicking hallmark components of OSA lead to the emergence of the morbid phenotypes that are characteristically encountered in patient with OSA. These morbidities appear to involve epigenetic processes (Figs. 1-3). Depending on the developmental stage of the occurrence of OSA, the putative role of such epigenetic changes may differ. In early onset OSA (i.e., gestational or postnatal), the epigenetic modifications may not impose immediate morbid consequences but may also promote gene alterations that will become manifest only much later in life, i.e., early antecedents of adult disease. When OSA occurs later in life, i.e., when the epigenetic window is less susceptible, occurrence of selected epigenetic changes in relevant genes and pathways may dictate the potential for reversibility of the morbid consequences of OSA. Hopefully, these concepts of uninterrupted continuum in disease, whereby diseases traditionally associated with older age should in reality be viewed as starting in childhood or even in foetal life will prompt future research efforts in these directions.

In addition, based on the evidence presented here, it is conceivable that with increased duration of disease, OSA will be accompanied by a gradually diminished reversibility of relevant morbid phenotypes. If such assumptions are indeed accurate, we should conceivably redesign our approaches to randomised controlled therapeutic trials in OSA by either taking into account antecedent disease duration (a virtually impossible challenge), or down estimating the effect sizes of therapeutic interventions and the predicted beneficial outcomes of such treatments, while adequately increasing cohort size needs for appropriately powered studies.

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CONFLICTS OF INTEREST

The author declares no conflict of interest.

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