Neurobiology

# Myelination and *APOE4* risk variant in Alzheimer's

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People who carry a particular variant of the *APOE* gene are at increased risk of developing Alzheimer's disease. It emerges that this might be due to decreased production of a fatty substance called myelin by oligodendrocyte cells. See p.769

Oligodendrocytes are cells of the central nervous system that produce myelin — a lipid-rich, multilayered membrane that wraps around the cable-like extensions of nerve cells, allowing efficient insulation, rapid conduction of impulses and metabolic support. In diseases such as multiple sclerosis, a temporary or permanent loss of myelin translates into bouts of illness. But the changes seen in oligodendrocytes and their precursors in both multiple sclerosis[1] and Alzheimer's disease[2–8] suggest that these cells might also contribute directly to the onset or progression of disease. On page 769, Blanchard *et al.*[9] unveil a previously unknown role for oligodendrocytes in Alzheimer's disease that involves the lipid carrier protein apolipoprotein E (APOE) and changes in cholesterol metabolism.

Oligodendrocytes must acquire a substantial store of building blocks to make the many layers of the myelin sheath needed to properly insulate neurons. This process involves the uptake, synthesis and translocation of lipids — a process facilitated partly by APOE, which serves as a link between various cell receptors for lipid uptake and lipids, such as cholesterol.

In humans, APOE is encoded by the *APOE* gene, which can exist as different variants. The protein translated from the *APOE4* variant differs from the *APOE3* product in the substitution of one amino-acid residue. But this simple change renders APOE4 dysfunctional[10], and people who carry *APOE4* are more susceptible to developing Alzheimer's disease than are those who do not. Exactly how *APOE4* contributes to disease progression is unclear, although several mechanisms have been proposed[10]. Conventionally, the disease has been considered a disorder of the grey matter — a brain region with low levels of myelination. But neuronal cell bodies that reside in the grey matter project axons into white matter, where myelination is more prominent. So the possibility that APOE4's role in Alzheimer's disease involves oligodendrocytes and myelination seems biologically plausible.

Blanchard and colleagues analysed the brains of 32 people after death, 20 of whom had been diagnosed with Alzheimer's disease. The total group included 20 *APOE4* carriers. The researchers used a technique called single-nucleus transcriptional profiling to define differences in gene expression between the groups. In *APOE4* carriers, they identified several biological pathways that were affected in various cell types, including oligodendrocytes. Interestingly, they found that a set of genes responsible for cholesterol biosynthesis and transport was dysregulated in oligodendrocytes. The individuals affected were either *APOE4* carriers or had shown signs of Alzheimer's disease. The authors then analysed the lipids and tissues of the brains' hippocampus and prefrontal cortex. They found that the cholesteryl ester species tended to accumulate in *APOE4* carriers (although further assessment using a larger sample size will be required to confirm the precise lipid species affected). This could indicate that cholesterol is not being properly incorporated into myelin in the context of *APOE4*.

Next, the researchers assessed the effects of *APOE4* on oligodendrocytes. They generated human oligodendrocytes from a type of stem cell called induced pluripotent stem cells that were engineered to carry either *APOE4* or *APOE3* but that had otherwise identical genetic backgrounds. In line with their findings in the brain tissue, the authors discovered a greater accumulation of cholesterol in oligodendrocytes carrying *APOE4* than in those carrying *APOE3* (Fig. 1). This accumulation occurred mainly in and around a subcellular compartment called the endoplasmic reticulum (ER), which is involved in protein and lipid synthesis and transport. The build-up of cholesterol caused ER stress and resulted in a stress-activated transcription factor (ATF6) moving to the nucleus. Notably, the authors' initial gene-expression profiling had predicted the occurrence of ER stress.

Blanchard and colleagues found that selected gene transcripts linked to myelination were downregulated in the brains of *APO4* carriers compared with *APOE3* carriers. Intriguingly, some genes were affected even in *APOE4* carriers who had shown no overt signs of Alzheimer's disease, suggesting that *APOE4*-mediated oligodendrocyte dysfunction

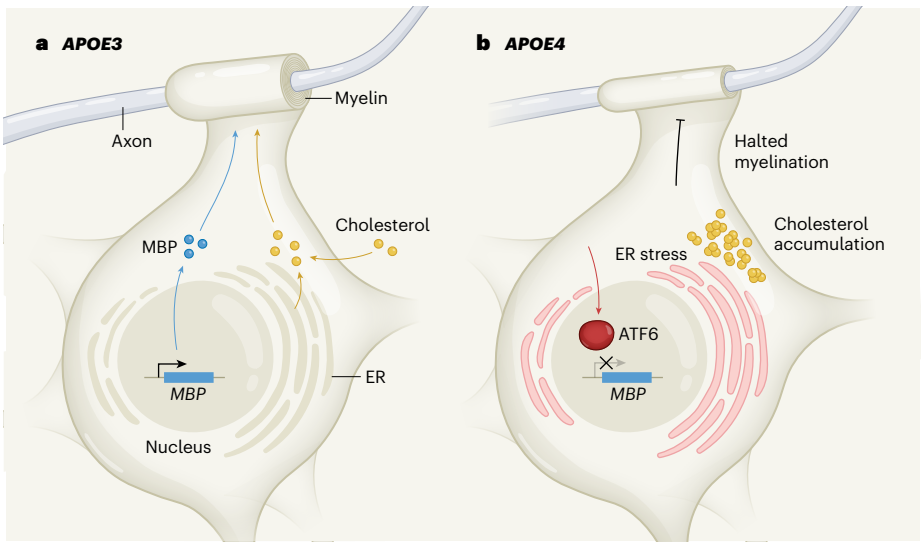


**Figure 1 | Effects of *APOE*-gene variants on myelin synthesis. a**, In people who carry a gene variant dubbed *APOE3*, cells called oligodendrocytes both synthesize cholesterol in the endoplasmic reticulum (ER) and import it. Expression of the gene myelin basic protein (*MBP*) leads to the production of MBP proteins, which combine with cholesterol and other biomolecules to make myelin sheaths that wrap around neuronal projections called axons. **b**, Blanchard *et al.*[9] find that, in people who harbour a different *APOE* variant called *APOE4*, cholesterol transport in oligodendrocytes is defective. Cholesterol accumulates in the cells, causing ER stress, and leading to movement of the transcription factor ATF6 to the nucleus. Levels of MBP decrease (although tit is unclear if this is related to ATF6), and these factors combine to reduce myelin synthesis. This might help to explain why people who carry *APOE4* are more likely to develop Alzheimer's disease than are those who carry *APOE3*.

might occur before clinical onset and in healthy people. Next, the authors asked whether the dysfunctional cholesterol transport seen in *APOE4* carriers interferes with the production of myelin by oligodendrocytes. Indeed, levels of myelin basic protein (MBP) — a component of myelin that is produced by oligodendrocytes as they differentiate — were decreased, as was the thickness of the myelin sheath in several *APOE4* carriers compared with *APOE3* carriers (Fig. 1). The group confirmed these findings both *in vitro* and in a mouse model of disease.

Finally, the authors addressed the question of whether the drug cyclodextrin — which facilitates cholesterol transport — could reduce the accumulation of cholesterol so as to favour the incorporation of cholesterol into myelin instead. Cyclodextrin indeed decreased the accumulation of cholesterol, increased the production of MBP and increased myelination, both *in vitro* and *in vivo*. What's more, cyclodextrin led to mild but significant improvements in learning and memory in *APOE4*-expressing mice.

That cholesterol is a limiting factor for oligodendrocyte maturation and myelin production has been suggested previously[11]. But by building their experiments on human gene-expression data, Blanchard *et al.* have shed light on how these cells might, in turn, contribute to the onset or progression of Alzheimer's disease. The fact that the authors also identified dysfunctional myelination at the transcriptional level in symptom-free *APOE4* carriers is interesting and extends a previous study[12] showing that myelin levels are low in infants who carry *APOE4*.

It remains unclear, however, whether the effects of APOE4 on oligodendrocytes are direct or exerted indirectly through surrounding cell types[13,14] (not least, brain-specific immune cells called microglia, in which APOE4 also affects lipid accumulation[15]). Although genome-wide association studies have highlighted microglia as a likely cellular effector in Alzheimer's disease[13,14], the new results suggest that variants such as *APOE4* might contribute by operating in other cell types or through alternative cellular mechanisms.

One might speculate that oligodendrocytes are under high metabolic pressure because their cell bodies and lipid bilayers expand dramatically to form myelin sheaths. This process demands efficient orchestration of the intracellular transport of metabolite molecules (including cholesterol), which might be compromised in *APOE4* carriers, contributing to disease onset. Our knowledge of metabolic regulation during oligodendrocyte differentiation and maturation is still limited, but Blanchard and colleagues' findings suggest that a route to a better understanding of Alzheimer's disease might involve looking for mechanisms that precede symptoms but that contribute to later pathology. Finally, although the improvements in learning and memory induced by cyclodextrin are mild, this study paves the way to possible treatments that target oligodendrocytes in Alzheimer's disease. Such treatments — together with drugs that target other mechanisms, such as the accumulation of insoluble Tau and amyloid-β protein plaques, which are considered hallmarks of Alzheimer's disease — might enable us to better tackle this devastating condition.

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## In retrospect

# 25 years of the segmentation clock gene

**Ryoichiro Kageyama**

The discovery of a gene that mediates periodic segmentation of the developing backbone of vertebrate embryos opened up research into how the pace of development is controlled by a molecular clock that has a species-specific rhythm.

It is a wonder to observe embryos developing autonomously under a seemingly predetermined schedule. Particularly striking is the developmental process by which structures called somites (which will differentiate into segmental body structures such as vertebrae, ribs and skeletal muscles) increase in number in a periodic manner. Twenty-five years ago, writing in *Cell*, Palmeirim *et al.*[1] reported the first molecular evidence of a gene linked to the periodicity of somite formation. The discovery paved the way to our understanding of the developmental clock — a network of genes whose expression oscillates synchronously and thereby regulates the timing of developmental events.

Somites form from an embryonic tissue called the presomitic mesoderm (PSM), which grows towards the caudal (tail) end of the embryo. As it grows, the rostral (head) end of the tissue segments repeatedly, and new segments are generated with a periodicity that is species-specific — once every 30 minutes in zebrafish, 90 minutes in chickens, 2 hours in mice and 5 hours in humans[2]. Somite formation has been actively studied since the nineteenth century, and the periodicity of this process led to the hypothesis that it was controlled by a cellular oscillator dubbed the clock[3]. However, despite researchers' fascination with this phenomenon, the underlying mechanism remained unknown.

By the mid-1990s, many genes that regulate body segmentation had been identified in fruit flies, but most of their vertebrate equivalents — known as homologues — were found not to be involved in somite formation[4]. By contrast, vertebrate homologues of fruit-fly genes such as *Notch*, which is involved in the development of neurons (but not in segmentation) in fruit flies, were shown to be involved in somite formation in mice[5]. This led researchers to ponder whether segmentation arose independently in flies and vertebrates. But a gene called *hairy*, which is involved in segmentation of the body in fruit flies[6], caught the attention of Palmeirim and colleagues. The group reasoned that, although somite segmentation is very different in vertebrates and invertebrates, the same gene might be involved.

Indeed, when the authors examined a homologue of *hairy* in chicks, *c-hairy1*, they found