# Epigenetics of *SNCA* and *APOE* Genes and Lewy Body Dementia

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Lewy Body Dementia (LBD) is a neurodegenerative disease characterized by rapid cognitive decline, Parkinson's-like motor issues, hallucinations and eventually death. LBD is suspected to be caused by the buildup of Alpha-Synuclein proteins. Though scientists are still unsure of Alpha-Synuclein's exact role in a healthy subject, it is possible that it may be responsible for transport vesicles in the brain and regulation of neurotransmitters. However, when it comes to diagnosing LBD, relying on the presence of protein aggregates to diagnose patients has proven to be fairly inaccurate. This is because LBD displays traits similar to other neurodegenerative conditions, like Alzheimer's disease (AD). One of these traits is the presence of Alpha-Synuclein proteins. So, while this protein is a decent indicator of the disease, understanding the concentration of this protein that may be associated with LBD along with the underlying mechanisms that cause it to arise in LBD subjects may help doctors improve their diagnostic criteria. Two genes have been studied relating to the development of LBD: *SNCA* and *APOE*. The normal function of *SNCA* is to produce the Alpha-Synuclein protein associated with diseases such as LBD. The typical function of *APOE* is to create the proteins that fuse with lipids to make lipoproteins. These lipoproteins then move lipids around the body via the bloodstream. Though only one of these genes is directly related to the Alpha-Synuclein protein, both have been linked to the development of LBD through epigenetic modifications. One of these modifications is hypomethylation, or undermethylation. Hypomethylation is when a 5-methylcytosine nucleotide loses its methyl group. and often creates instability due to the random shuffling of chromosomes. Hypomethylation in *SNCA* and *APOE* genes is positively correlated with developing Lewy Body Dementia, which demonstrates these genes could be potential biomarkers to diagnose LBD if their mechanistic contribution to LBD development is better understood.

AD and LBD are two of the most common neurodegenerative dementias, with LBD making up 15% to 30% of all neurodegenerative dementias (Sanghvi et al., 2020). Although both diseases

have some characteristics that set them apart, such as LBD having Parkinson's-like movement issues and a higher mortality rate than AD (Sanghvi et al., 2020), these distinct symptoms tend not to arise until the disease has begun to take its degenerative course. Even though these diseases have a few factors that set them apart, doctors still have difficulty when diagnosing patients, as most of the symptoms, such as tauopathies, hallucinations and olfactory disorders tend to overlap between both conditions (Foguem et al., 2018). Therefore, research into diagnosing LBD as early as possible has become critical to ensure proper treatment is administered. Recent studies have singled out some candidate genes that could serve as potential biomarkers for LBD. It is hypothesized that one of these genes, *SNCA*, typically functions to assist in the regulation of dopamine transport, induce fibrillization of tau proteins and assist in the production of Alpha-Synuclein proteins (Siddiqui et al., 2016). Alpha-Synuclein proteins and dopamine have been linked to neurodegeneration when their normal interactions are altered (Mor et al., 2019), which could suggest that modifying *SNCA* function may modify these interactions, potentially increasing the likelihood of developing LBD. Additionally, recent studies have even suggested that Alpha-Synuclein levels could be used to differentiate between AD and LBD (Lilamand et al., 2022). Another gene, *APOE* (specifically at its e4 allele), is hypothesized to assist in the production of lipoproteins (Tulloch et al., 2018). However, unlike *SNCA*, its function in relation to the development of LBD remains unclear, as it is still being studied. Regardless, the studies conducted by Funahashi et al. and Tulloch et al., suggest that the hypomethylation of these two genes may impact their function in a way that increases the chances of developing LBD.

Because LBD is harder to diagnose due to its many shared characteristics with other neurodegenerative diseases such as Parkinsons or Alzheimer's, LBD is often *misdiagnosed* as AD. Lilamand et al. sought to find a more reliable strategy to diagnose LBD. They created three groups, selecting subjects that met the diagnostic criteria of LBD, subjects that served as neurological

controls (NC), and subjects that met the diagnostic criteria of Alzheimer's Disease (AD). Blood samples and neurological screenings were performed and then followed by the collection of a cerebrospinal fluid (CSF) sample. After the collection of the CSF samples, they analyzed the levels of Alpha-Synuclein proteins present in each sample (Lilamand et al., 2022). The data showed that the average amount of Alpha-Synuclein present in LBD subjects was about 1.28 ng/mL, while the average Alpha-Synuclein concentration in AD subjects was about 2.26 ng/mL ($p < 0.001$). These results suggest that the average amount of Alpha-Synuclein present in LBD subjects was significantly lower than those with AD (Lilamand et al., 2022). With these results, they can conclude that Alpha-Synuclein levels have the potential to be used as an LBD biomarker. Because the presence of Alpha-Synuclein significantly differs between these two diseases, this difference can be used to assess whether a patient has AD or LBD during diagnosis. Although Alpha-Synuclein levels alone cannot predict the condition of a patient, this value can help doctors find the distinction between AD and LBD. Improving the ability to discriminate between these two diseases with biomarkers such as Alpha-Synuclein can help decrease the likelihood of misdiagnosis in patients with LBD, in turn leading to better treatment outcomes.

The buildup of Alpha-Synuclein proteins, which causes synaptic dysfunction in patients, is considered a common indicator of LBD formation. The link between this protein and LBD could implicate *SNCA*, the gene thought to be responsible for Alpha-Synuclein synthesis, in the formation of LBD as well. Funahashi et al. investigated to see if epigenetic modifications of *SNCA* had any involvement in the development of LBD. In order to determine whether epigenetic changes at the *SNCA* gene contributed to LBD, Funahashi et al. (2017) collected blood samples from 20 LBD patients and 20 healthy controls. Using these samples, DNA was then processed with Bisulfite conversion and pyrosequencing in order to target a specific CpG-rich region of *SNCA* intron 1. They then observed DNA methylation at 10 different CpG sites found in this region using PCR. The data

shows that DNA methylation rates in subjects with LBD are significantly lower ($p < 0.001$) than healthy subjects (Funahashi et al., 2017). This is especially evident at CpG site 4, with the healthy control subjects having an average methylation rate 36% larger than the average methylation rate of LBD subjects. This difference between LBD and control subjects was also observed in the overall average of all the CpG methylation rates, with the methylation rates of the control subjects being 25% greater than those of LBD subjects. These results indicate that there may be a link between LBD and hypomethylation of the *SNCA* gene. The findings of this study imply that reduced methylation rates of *SNCA* could serve as a potential biomarker when diagnosing DLB. These findings also strengthen the data obtained by Lilamand et al. in that they offer an explanation as to why the build up of Alpha-Synuclein is more prevalent in LBD patients. Both studies discuss how Alpha-Synuclein aggregates can serve as reliable biomarkers for LBD, but only Funahashi et al. discusses how the gene responsible for Alpha-Synuclein production can be modified to produce irregular protein aggregates. Although *SNCA* is one of the better studied genes involved with LBD, it will take further research into how exactly hypomethylation impacts *SNCA* and Alpha-Synuclein production before it can be used to formally diagnose patients (Funahashi et al., 2017).

*SNCA* is not the only gene suspected to be associated with the formation of LBD. Multiple studies have found that inheriting the *APOE* gene, specifically its e4 allele, increases the chances of both AD and LBD arising in an individual. However, these studies don't offer any specific explanations about how this gene, its allele and function are associated with the prognosis of LBD. Tulloch et al. wanted to answer the question of how methylation may impact the *APOE* gene in relation to the formation of LBD. They performed bisulfite pyrosequencing on postmortem brain tissue samples from four separate groups. These four groups included five healthy subjects or controls, six subjects with AD, 12 subjects with LBD and 12 subjects with both AD and LBD. After the tissues were collected, 500 ng of genomic DNA was isolated from the frontal lobe and

cerebellum tissue of each subject and then bisulfite converted. DNA methylation levels were then observed across the samples using PCR (Tulloch et al., 2018). The results of this study displayed that only the frontal lobe samples displayed significant variation in DNA methylation levels, with no DNA methylation variation present in the cerebellum samples. In these frontal lobe samples, LBD subjects exhibited significantly lower average levels of DNA methylation (82.3%) compared to average levels of DNA methylation observed in control subjects (87.6%) ($p < 0.001$). Although the LBD subjects didn't have the lowest methylation levels, with the LBD + AD group demonstrating the lowest out of all the subjects with average DNA methylation levels of 78.7%, the LBD subjects still exhibited a noticeable reduction in methylation (Tulloch et al., 2018). These results indicate that there may be a link between LBD and hypomethylation of the *APOE* gene. Because a significant decrease in *APOE* methylation rates was found in subjects with both AD and LBD, and then followed by LBD alone, this implies that the *APOE* gene, specifically at the e4 allele, could perhaps act as a biomarker for those afflicted with LBD and AD or just LBD. These results, along with those of Funahashi et al., suggest that the hypomethylation of a certain gene could indicate the likelihood of developing LBD. Although Funahashi et al. explores the hypomethylation of *SNCA* and Tulloch et al. explores the hypomethylation of *APOE*, the results of both studies support the conclusion that the hypomethylation of their respective gene could serve as a possible biomarker for LBD. However, it will take further research into the exact function and purpose of the *APOE* gene before it can be incorporated into LBD diagnostic criteria.

Creating a solid diagnostic criterion of LBD has proven to be difficult, as this disease is multifaceted and manifests in the patient in both physical and behavioral ways. However, recent breakthroughs have found that hypomethylation of certain genes may be responsible for the development of LBD. One of these genes, *SNCA*, is responsible for Alpha-Synuclein synthesis. The buildup of this protein has been positively correlated with neurodegenerative diseases such as LBD

(Sanghvi et al., 2020). Additionally, studies have shown that hypomethylation of the *SNCA* gene may contribute to the overproduction of this protein, demonstrating that *SNCA* hypomethylation may be positively correlated with LBD (Funahashi et al., 2017). Another gene, *APOE*, has also been linked to the development of LBD. Although the gene's exact function in the body and how it relates to LBD has not been studied as thoroughly as *SNCA*, hypomethylation of *APOE* has been positively correlated with a higher risk of LBD (Tulloch et al., 2018). Overall, LBD is hard to diagnose due to its unclear molecular causes and because of its similarities to more well-known diseases like AD. Even though there are currently multiple genes considered to have significant association with the development of LBD, such as *APOE* and *SNCA* (Sanghvi et al., 2020), the connection between gene and disease still needs further research to solidify the candidacy of these genes as LBD biomarkers. More specifically, more genome-wide association studies (GWAS) should be conducted to establish stronger relationships between potential biomarker genes and LBD risk (Sanghvi et al., 2020). Further GWAS could also find more genes related to the formation of this disease. In the end, finding reliable LBD biomarkers is most beneficial to LBD patients and their healthcare professionals. The ability to detect LBD development along with improved, specific diagnostic criteria can help reduce the chances of misdiagnosis in patients with LBD. Increasing proper diagnosis in LBD patients is incredibly important, as it helps both patient and doctor when discussing personalized treatment and care, giving both a better idea of what to expect from the disease prognosis.

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