A Comprehensive Review and Evaluation of the Diagnostic Methods of a Multiple System Atrophy Diagnosis

Ria Nabar

Abstract:

Multiple System Atrophy (MSA) is a rare neurodegenerative disorder belonging to a group called synucleinopathies. It is characterized by the abnormal accumulation of α-synuclein in oligodendrocytes, causing glial cytoplasmic inclusions. MSA is commonly misdiagnosed due to its overlapping symptoms with other diseases, namely Parkinson's Disease (PD). This study reviews the diagnostic methods used for MSA and discusses their benefits and how they can be improved for future research. These methods include the current diagnostic criteria, MRIs, biomarkers, and genetic screening. Through a comprehensive search of the current literature, utilizing PubMed and Google Scholar, I analyzed and compiled numerous research articles for their relevance and content. Emphasizing the accurate and distinctive diagnosis of MSA allows individuals to better manage the consequences of this devastating disease.

Introduction:

Multiple System Atrophy (MSA) is a rare adult-onset neurodegenerative disorder with no known cause or treatment [1]. It belongs to a group of neurodegenerative disorders called synucleinopathies, which include Parkinson's Disease (PD) and Dementia with Lewy Bodies (DLB). The pathological hallmark of MSA is the abnormal accumulation of the α-synuclein protein in oligodendrocytes, which are glial cells that produce myelin in the central nervous system [1, 2].

MSA is divided into two main categories: the Parkinsonian-type (MSA-P), characterized by motor abnormalities, and the Cerebellar-type (MSA-C), characterized by a lack of coordination [2]. MSA is often misdiagnosed due to its overlapping symptoms with other disorders, especially Parkinson's Disease (PD). The disorder presents with symptoms of autonomic failure, parkinsonism, and cerebellar dysfunction [4]. Autonomic failure occurs when the autonomic system fails to regulate involuntary bodily functions, such as heart rate and blood pressure. Parkinsonism is a condition of rigidity, slow movements, and tremors, commonly associated with PD. Cerebellar dysfunction refers to difficulties in maintaining balance and performing coordinated movements.

MSA affects many regions of the brain, the most significant being the cerebellum (Figure 3), due to its role in movement and balance. The middle cerebellar peduncle is responsible for relaying information from the cerebrum and pons to the cerebellum, as well as for motor skills and movements. Another major brain region affected by MSA is the brainstem (Figure 2), which includes the midbrain, pons, and medulla. The pons and medulla are more affected by MSA than the midbrain; the pons regulates unconscious processes such as sleeping and breathing and the medulla regulates other autonomic processes such as heart rate, circulation, and blood pressure. The putamen, a structure in the basal ganglia, is also affected by MSA and is responsible for motor control and learning.

Methods of diagnostic criteria, MRI imaging techniques, biomarkers, and genetic screening have been investigated to improve the diagnosis of MSA. Diagnostic criteria provide a set of signs and symptoms that guide a patient's diagnosis. MRI (magnetic resonance imaging) is an imaging technique that provides images of the anatomy and structure of the body. Biomarkers are molecules found in the body that help indicate the presence of a disease.

Genetic screening involves identifying genes that may increase the risk of disease. Through my research of these different methods, I explore how the medical field can improve the diagnostic criteria in order for MSA to be more distinguishable from other neurodegenerative diseases. Based on current findings, appropriate diagnostic measures, that accurately identify MSA and differentiate it from other diseases, should be emphasized to help improve the diagnosis of the disease.

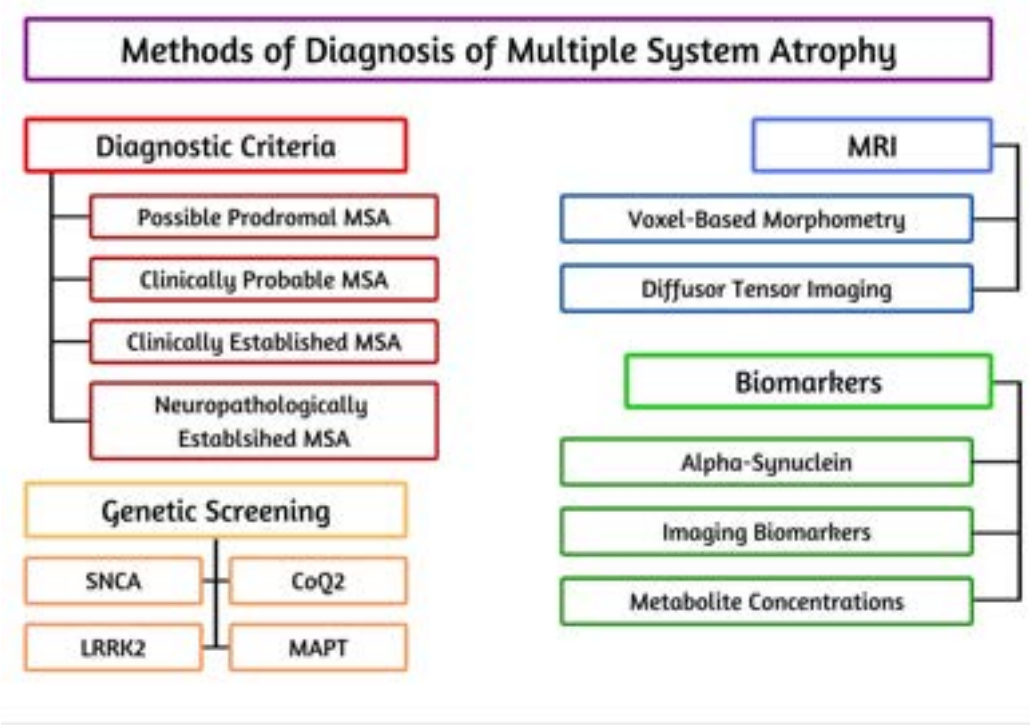

Figure 1. Summary of Diagnostic Methods. Summary of the different methods used to diagnose MSA that were discussed in this paper: diagnostic criteria, MRIs, genetic screening, and biomarkers.

Methods:

I conducted a comprehensive search of the current literature utilizing PubMed and Google Scholar. In my search I compiled around 50 research articles for their content of Multiple System Atrophy and diagnostic techniques. After reviewing the papers in depth, I narrowed down my references to 36 research articles which I believed to be most relevant to my paper.

Additionally, I took the MRIs, of brain structures of MSA and PD patients and health controls, that I referenced and created simplified drawings to emphasize the differences between the respective characteristics [4]. I highlighted these images because they displayed the most significance in distinguishing diagnosis.

Diagnostic Criteria:

Having reliable and valid guidelines for diagnostic criteria is a crucial aspect of correctly diagnosing MSA since MSA presents with symptoms that are very similar to other disorders, especially PD. The Movement Disorder Society outlines four levels of diagnostic certainty of MSA: possible prodromal MSA, clinically probable MSA, clinically established MSA, and neuropathologically established MSA [4]. The different levels allow clinicians to manage the disease appropriately and pursue an approach best suited for the severity of the disease. The primary features necessary for a clinical MSA diagnosis of any level are the onset of symptoms after 30 years of age and an increase in disease severity over time. The presence of a negative family history is also a factor as MSA is generally a sporadic, not inherited disease, but there have been rare cases of familial MSA [29, 30]. More often than not, if there is a history of other family members having the same disorder, it is likely not MSA but rather another neurodegenerative disorder such as PD.

Possible prodromal MSA is an early stage of MSA where symptoms are not fully present, but there are signs of disease development. These signs tend to present as subtler, less severe symptoms than the core clinical features of clinically established MSA. Identifying this stage allows for earlier intervention and management of the disease. Further research is being conducted to develop treatments and slow disease progression [4].

Core clinical features of clinically probable and clinically established MSA include urogenital failure, cardiovascular autonomic failure, parkinsonism, and cerebellar syndrome [4]. Urogenital failure refers to bladder control problems, sexual dysfunction, and kidney failure. Cardiovascular autonomic failure is caused by autonomic nervous system dysfunction. Other supportive features of clinically established and clinically probable MSA are the increase in MSA symptoms over time and postural instability. Structural changes in the brain, evaluated through MRIs, also signify MSA. This includes atrophy of the pons (Figure 2a), putamen, middle cerebellar peduncle (Figure 3a), and cerebellum (Figure 2a, Figure 3a), as well as the hot cross bun sign in the pons (Figure 4a), described in further detail as a biomarker [4]. Clinically probable MSA requires fewer symptoms and features to be present than clinically established MSA. These symptoms are more significant than those of possible prodromal MSA but do not yet meet the full criteria for clinically established MSA. Differences include more supportive features, the presence of MRI markers, and greater diagnostic certainty in clinically established MSA compared to clinically probable [4].

Neuropathologically established MSA refers to definitively diagnosing MSA through brain tissue examination postmortem, or after death. Key features involve the abnormal accumulation of the protein α-synuclein in neurons and glial cells and striatonigral and olivopontocerebellar degeneration [4]. Striatonigral degeneration decreases basal ganglia size, resulting in rigidity and bradykinesia, or slow movement. Olivopontocerebellar degeneration refers to atrophy of the olivary nucleus, cerebellum, and pons, leading to difficulties with balance and muscle coordination. This postmortem analysis supports the diagnosis of MSA and provides further information on the disease's progression.

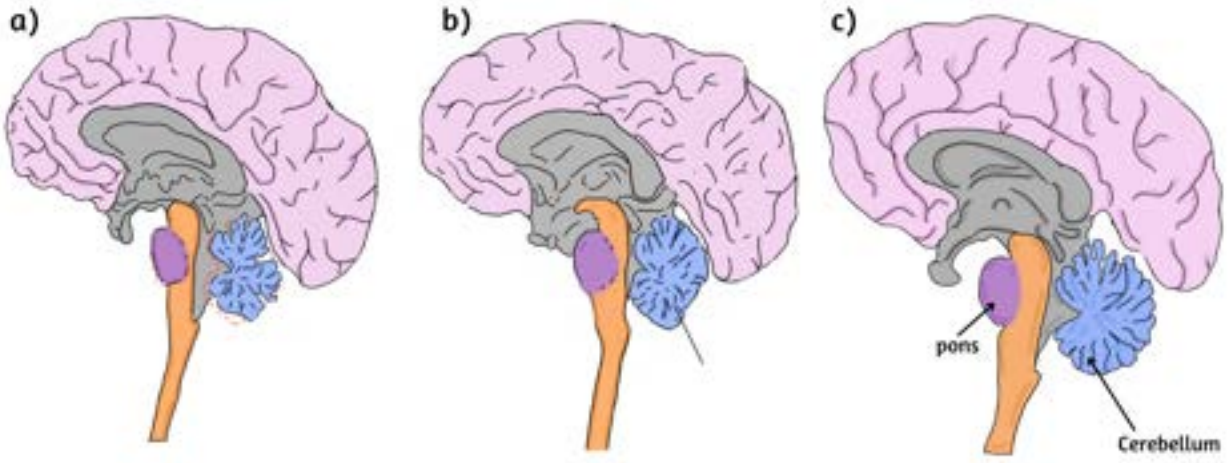

Figure 2. Representation of pons and cerebellum in MSA. The midsagittal view of atrophy in the pons and cerebellum in an MSA patient (a) compared to little atrophy in a PD patient (b), and a healthy brain (c). The red dotted lines compare an MSA pons and cerebellum to a healthy pons and cerebellum [4]. (These figures are not drawn to scale and serve purely as image representations).

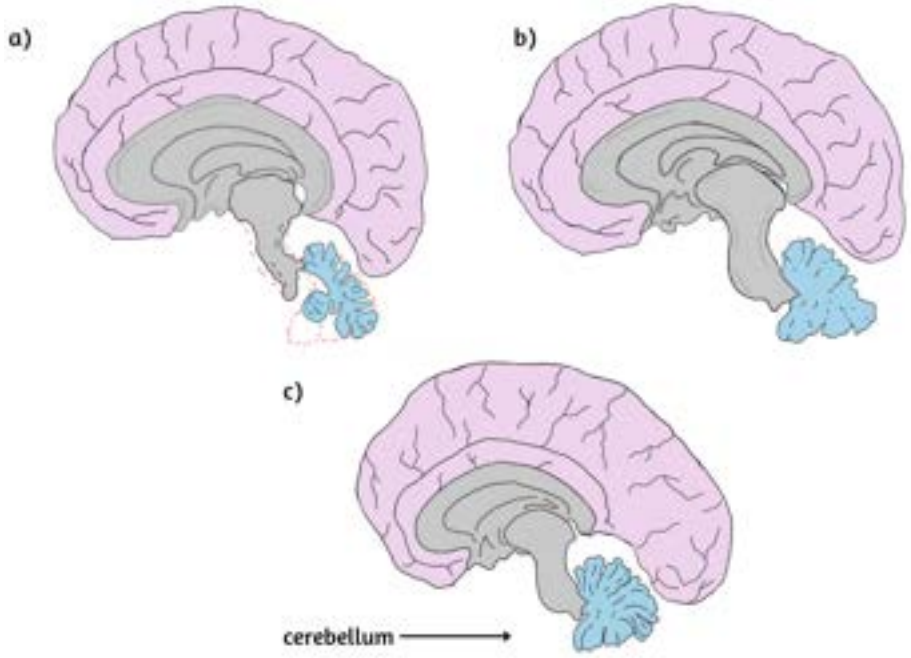

Figure 3. Representation of cerebellum in MSA. The parasagittal view of a closer look at cerebellar atrophy, specifically the middle cerebellar peduncle. The MSA patient (a) is seen to have significantly more atrophy than the PD patient (b), and both are compared to a healthy brain image (c). The red dotted lines indicate the comparison of an MSA cerebellum to a healthy cerebellum and the PD cerebellum is less defined to represent the loss of neural pathways [4]. (These figures are not drawn to scale and serve purely as image representations).

MRI

Magnetic resonance imaging (MRI) is a technique that provides images of the anatomy and structure of the body using a magnet and radio waves. Recent studies have been conducted using different MRI techniques to distinguish MSA from PD. Two techniques, voxel-based morphometry (VBM) and diffusor-tensor imaging (DTI) have been effective in providing changes in brain structure associated with MSA [6, 7, 8].

Voxel-based morphometry [7] is used in MRIs to measure the volume and density of different brain structures, especially highlighting differences in tissue loss between gray and white matter. Gray matter consists of cell bodies, and white matter consists of myelinated axons. VBM is often used to detect pathological changes within the brain and is useful when focusing on gray matter specifically. One study used VBM to analyze MRIs of 14 MSA patients versus those of 13 healthy control (HC) subjects [7]. The study emphasized how VBM shows the localization of atrophy and loss of brain tissue in gray and white matter. The results displayed a significant reduction of gray and white matter in the cerebellum and brainstem. Of the brain structures analyzed, the middle cerebellar peduncle exhibited the most shrinkage through extreme loss of white matter. Shrinkage of the middle cerebellar peduncle negatively affects communication between brain structures responsible for motor skills.

While this study did not compare images of MSA patients to PD patients, a different study compared cortical atrophy between the two [19]. VBM was used to analyze the gray and white matter of 12 probable MSA-P patients, 12 PD patients, and 12 healthy control subjects. Results showed significantly greater atrophy of the cortex and subcortex in MSA-P patients compared to PD patients. Atrophy in MSA-P patients tended to be spread throughout various cortical and subcortical areas, while atrophy in PD patients was localized to the left side, specifically the left region of the basal ganglia [19]. To generalize these findings to greater populations, studying larger sample sizes would be beneficial to ensure that these results are consistent to a certain extent.

Diffusion tensor imaging (DTI) is an MRI technique that detects the rate of diffusion of water molecules to provide information about the density of white matter in structures of the brain. Measuring the diffusion of water molecules is necessary because, in a healthy brain, diffusion is restricted by brain structures. As brain structures begin to atrophy, water molecules diffuse with greater ease in different directions. DTI provides further detail on the severity of atrophy and is often used when studying communication between brain structures.

One study researched differences in mean diffusivity (MD), the average rate at which water molecules diffuse, and fractional anisotropy (FA), which measures the directional restriction of water movement in brain tissue. The study involved 28 MSA patients, 19 PD patients, and 25 healthy control subjects [6]. The results showed increased MD values and decreased FA values in MSA patients than those of PD patients and healthy controls. Higher MD values suggest an increase in water movement, indicating tissue damage and loss of brain structure. Lower FA values suggest that water moves in all directions, rather than in a certain direction, indicating brain tissue damage. The most impacted regions of the brain were the cerebellum white matter, cerebellar cortex, putamen, and middle cerebellar peduncle. The notable differences between MSA and PD patients show the value of this technique in differential diagnoses.

Another DTI study, of 20 MSA patients (10 MSA-C and 10 MSA-P) and 20 healthy control subjects, researched methods of fractional anisotropy and mean diffusivity, as well as axial diffusivity and radial diffusivity [20]. Axial diffusivity (AD) measures the diffusion of water

molecules moving parallel to the direction of nerve fibers. AD values indicate the health of these nerve fibers; high values indicate greater nerve fiber health, allowing water to move along the fibers, and low values indicate damage to the nerve fibers, causing water movement to be restricted. Radial diffusivity (RD) measures the diffusion of water molecules perpendicular to the direction of nerve fibers. RD values indicate the health of the myelin sheath that protects the nerve fibers; low values mean that the myelin sheath is healthy, allowing less water to move in a perpendicular direction, and high values indicate a damaged myelin sheath, causing increased perpendicular water movement. Results of the study presented increased RD and MD values, and decreased FA values, in the cerebellar peduncles of MSA patients; AD values only increased slightly in the cerebellar peduncles of MSA patients [20]. The study explained that RD and MD values proved to be most helpful in identifying MSA due to the significant increase of the values in the bilateral putamen and middle cerebellar peduncle. These values all signify damage to the nerves and brain structures, except for, interestingly, the AD values that increased. RD and AD values should be further researched to explore the consistency of these results, as well as to compare the values of MSA and PD patients.

While DTI shows great promise in identifying MSA, it does require a high level of expertise and technology, limiting its availability. DTI uses high-field MRI scanners to produce images that are extremely detailed and accurate. Additionally, it requires specialized imaging sequences to accurately measure the diffusion of the water molecules, as well as advanced software to calculate and process the data received. A high level of expertise is necessary to operate the scanners, analyze and interpret the data, and apply the results to a clinical setting. To increase DTI accessibility, more affordable scanners should be developed through increased production, and education and training should be more widespread among technicians and researchers.

Biomarkers:

A biomarker is a molecule found in the body that suggests the presence of a disease. Identifying biomarkers of MSA allows for better diagnosis and differentiation of the disorder. Recent studies have found possibilities in fluids, gut and tissue microbiota, and imaging biomarkers [11].

A fundamental biomarker is α-synuclein [11], a protein, found in neurons and glial cells, that regulates neurotransmitter disease. When there is an abundance of α-synuclein in these cells, glial cytoplasmic inclusions (GCIs) are formed. This accumulation of α-synuclein impairs neuronal cellular functions and leads to neurodegeneration. A-synuclein is especially prominent in dopaminergic neurons, which release the neurotransmitter dopamine [14]. Dopamine is important in supporting movement and is therefore a crucial aspect of movement disorders like MSA and PD. When the aggregation of α-synuclein impairs neurons, it disrupts dopamine release, causing the motor symptoms seen in those disorders.

A-synuclein aggregates through the process of misfolding, where the protein takes on an abnormal shape. The misfolded forms clump together causing inclusions in the neurons. Protein misfolding cyclic amplification (PMCA) is a technique that amplifies this misfolding process to detect α-synuclein aggregates in biological fluids [5]. A study obtained cerebrospinal fluid (CSF) samples from 94 PD patients, 75 MSA patients, and 56 healthy control subjects. A-synuclein aggregates can be measured through CSF since neurodegeneration causes neurons to release α-synuclein into extracellular spaces of the brain and spinal cord. The study used a dye called thioflavin T (ThT), which tracks the aggregation by binding to the α-synuclein. Once it binds, it

displays an intense fluorescence which helps to quantify the aggregation. After α-syn-PMCA, the results showed significantly higher maximum fluorescence in the CSF samples of MSA patients compared to PD patients [5]. The study also determined that the α-synuclein aggregates in CSF reflected those in the brain, allowing for a less invasive way to detect the protein.

There are three main forms of α-synuclein: total α-syn (t-α-syn), phosphorylated α-syn (p-syn), and α-syn oligomers (o-α-syn) [11]. T-α-syn refers to all of the forms of α-synuclein and gives an overall measure of its abundance. P-syn is a type of α-synuclein that has been phosphorylated and is most closely associated with disease progression. O-α-syn is an aggregated form of α-synuclein that is highly toxic and plays a large part in disease mechanisms. Many studies have measured t-α-syn levels in cerebrospinal fluid (CSF). While some of them found no difference in levels between healthy controls and MSA patients, the majority of the studies found lower levels in MSA patients compared to those of healthy controls [11]. However, not much of a difference was found between the t-α-syn levels in MSA patients versus patients with other neurodegenerative disorders. Several studies have researched the value of phosphorylated α-syn as a biomarker. Measuring p-syn levels in CSF and red blood cells revealed elevated levels of p-syn in MSA patients than in PD patients and healthy controls [31-33]. The third form, o-α-syn, is a more reliable biomarker in red blood cells and brain samples than CSF. Increased levels of o-α-syn were found in MSA patients compared to healthy controls, and widespread accumulation of o-α-syn in brain samples was greater in MSA patients than PD patients [11, 34]. Based on these findings, p-syn seems to be the most promising in diagnosing MSA and differentiating between other neurodegenerative disorders.

A-synuclein is also present in organs and tissues such as salivary glands, skin, and the gastrointestinal tract. Many studies have shown that p-syn levels can be measured through skin biopsies as well. The majority of the results show that p-syn is detected in the skin biopsies of MSA patients but not in healthy controls [11, 35, 36]. Skin biopsies should be further explored and utilized more, as they are a valid and non-invasive way to test for MSA.

Imaging biomarkers, such as MRIs, have also been studied. The hot cross bun sign, located in the pons, and the putaminal slit sign, located in the putamen, have been found in the MRIs of many MSA patients (Figure a) [11]. The hot cross bun sign is caused by a loss of neurons and myelinated fibers in the pons, while the putaminal slit sign is caused by striatonigral degeneration. The value of these signs as biomarkers was evaluated through specificity and sensitivity. Specificity indicates the rate of false positives or how often patients are misdiagnosed as having the disease. High specificity suggests a low rate of false positives and low specificity indicates a high rate of false positives. Sensitivity measures the rate of correct diagnoses of those who have the disease. High sensitivity indicates a high rate of correct diagnoses and a low sensitivity indicates a low rate of correct diagnoses. The hot cross bun sign was found to have 100% specificity and 50% sensitivity, showing no false positives but some false negatives. The putaminal slit sign was found to have 90% specificity and 30% sensitivity, showing a low rate of false positives but a higher rate of false negatives. It should also be noted that the putaminal slit sign was present in many of the MRIs of healthy control subjects, limiting its value as a biomarker [11].

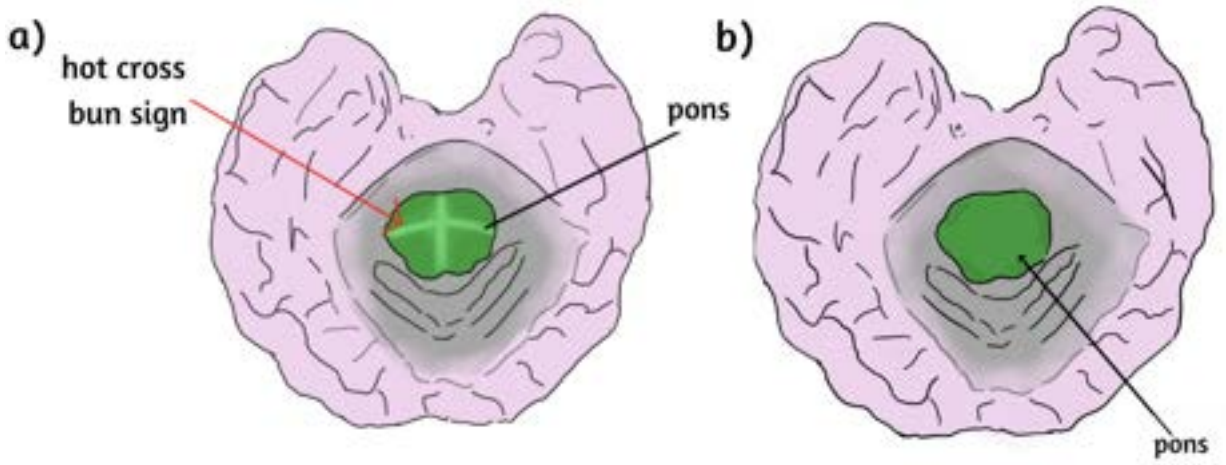

Figure 4. Representation of hot cross bun sign in MSA. Transverse view of an imaging biomarker, the hot cross bun sign in the pons of an MSA patient (a) compared to the pons in a healthy brain (b) [4]. (These figures are not drawn to scale and serve purely as image representations).

One study researched biomarkers that displayed the level of disease severity in MSA-C patients [10]. Proton magnetic resonance imaging, a technique used to measure metabolite concentrations, was used to image brain regions affected by MSA. The study measured concentrations of N-acetyl aspartate (NAA) and myo-inositol (MI). NAA is a metabolite involved in axon-glial signaling and reflects the health of neurons. MI is a sugar in the brain that is an indicator of how glial cells respond to injury of the nervous system. The study measured metabolite concentrations in the pons and the medulla of 12 patients with MSA-C and 12 healthy control subjects. The results showed significantly higher mean concentration levels of MI in MSA-C patients than in healthy control subjects in the pons ($P<0.05$) and medulla ($P<0.05$). Lower mean concentration levels of NAA were found in MSA-C patients compared to health control subjects in the pons ($P<0.05$) and medulla ($P<0.05$) [10]. The study confirmed the role of NAA and MI as biomarkers for clinical severity of MSA. However, it was odd that the cerebellum was not included in the study seeing as it plays a major role in MSA and has been the main focus in other studies. In the future, it would also be beneficial to include MSA-P and PD patients to study the extent to which these biomarkers can be used as distinguishing factors.

Genetic Screening

MSA is a sporadic disease that occurs randomly and without any distinguishable pattern. While it is rare for MSA to run in families, there may be possible genes that contribute to the disease. Understanding and identifying the genetic components of MSA can help better diagnose those who are at risk and develop future treatments.

The SNCA gene is one of the more prominent genes concerning MSA and is responsible for encoding the α-synuclein protein [3]. Studies have been conducted to evaluate mutations in the SNCA gene. The variants of SNCA can lead to α-synuclein aggregation and therefore cause the neurodegeneration and symptoms characteristic of MSA. Many variants have been present in European populations with MSA, but not in Asian ones [22-24]. A study of the rs11931074 variant of SNCA noted a strong association with MSA in Caucasian subjects, but none in Korean subjects [24]. The study further evaluated these findings within 96 Chinese MSA

patients and 120 healthy controls and came to the same conclusion as the study with the Korean participants. One European sample study evaluated 32 different SNCA mutations in 239 living MSA patients and 617 controls recruited from the UK, France, and Germany [13]. The controls consisted of the spouses of MSA patients in the UK and from DNA banks in France and Germany. The study found two mutations with the strongest association with MSA: rs3822086 and rs3775444 [13]. A replication study consisting of 78 MSA blood samples from a pathologically proven MSA brain bank confirmed these results. A further contradiction is presented by a genome-wide association study of 918 MSA patients of European descent and 3,864 health controls [23]. The patients were either clinically diagnosed with possible or probable MSA, or pathologically diagnosed with definite MSA. Even though this was a study of a European sample, no association between the SNCA gene and MSA was found. Testing of the SNCA gene is currently a disputed identifying factor of MSA within European populations and requires further research, and should also be further explored within other ethnicities.

MSA is characterized by neurodegeneration, and mitochondrial dysfunction contributes to this process. Mitochondria are located in cells and are essential for producing energy to support bodily processes. Impaired mitochondrial function can result in oxidative stress and lower energy production, causing damage to neurons. The gene coenzyme Q2 (CoQ2) creates coenzyme Q10 (CoQ10), which is essential for proper mitochondrial function and energy production [3]. Studies have found decreased levels of CoQ10 in MSA patients, thus possibly explaining the mitochondrial dysfunction. One study of an Italian population sample evaluated seven MSA-P patients, seven MSA-C patients, and six healthy control subjects through skin biopsies and DNA extractions from blood cells. [12]. The results showed reduced CoQ10 in fibroblast cells of both MSA-C and MSA-P patients. While the cause of these reduced levels is unclear, mutations of the CoQ2 gene may be a contributing factor. Another study of a Japanese sample, with 133 MSA patients and 200 healthy controls, found an association between another variant of CoQ2, L25V, and MSA [26]. Additionally, a study of Japanese, North American, and European populations identified other variants associated with MSA, among them the V343A variant commonly found in Japanese MSA patients [25]. However, studies have also contradicted the association of CoQ2 with MSA [22, 23, 28]. One study of a Chinese population sample of 312 MSA patients, found no association between the variant Val393Ala of CoQ2 and MSA [28]. The genome-wide association study that found no relation between SNCA and MSA consequently found no relation between CoQ2 and MSA in the European sample [23]. A vital factor to note among all these studies is the different variants of CoQ2 that were evaluated. To solidify these findings, researchers should study specific variants of CoQ2 across different ethnic populations in the future.

LRRK2 is a protein, primarily present in neurons and glial cells, that is commonly associated with Parkinson's Disease [3]. It regulates cellular processes, such as kinase activity, cell signaling, and neuronal function [15]. Kinase activity involves phosphorylating other proteins needed to regulate cell growth and autophagy pathways. Mutations in LRRK2 can significantly increase kinase activity, causing toxicity in neurons. This contributes to the development of neurodegenerative characteristics of PD. Due to the pathological overlap between PD and MSA, there may also be a possible association between LRRK2 and MSA. Since LRRK2 is present in glial cells, it may have a role in glial cytoplasmic inclusions, which are highly characteristic of MSA. The G2019S-LRRK2 mutation has recently been explored in two cases of MSA. One case involved a Caucasian male diagnosed with probable MSA-P at age 40 [16]. After his death at age 49, DNA from his blood and frontal cortex was examined and revealed the G2019S-LRRK2

mutation. This was the first case where MSA was associated with this mutation. The second case involved a Moroccan female diagnosed with MSA-P, who died at age 58 [17]. Genetic testing consequently revealed the G2019S-LRRK2 mutation. One study of a Chinese sample, consisting of 318 MSA patients and 350 health controls, examined the association of R1628P and G2385R variants with MSA [27]. These variants were previously found to be associated with PD in Chinese populations, but not identified as risk factors of MSA. There seems to be a stronger relationship between the G2019S-LRRK2 mutation than the R1628P and G2385R ones in MSA. Further research surrounding this gene must be explored to strengthen this relationship.

Tauopathies are neurodegenerative disorders, such as Alzheimer's disease, that involve the abnormal accumulation of tau within the brain. The microtubule-associated protein tau (MAPT) gene, is responsible for making the tau protein, essential in stabilizing cell structures and managing intracellular transportation [3]. While MSA is classified as a synucleinopathy because of its abnormal accumulation of α-synuclein, studies have found the MAPT gene to be in possible association with MSA. This is likely because the tau protein can interact with α-synuclein and can worsen disease progression by doing so. Tau is present in GCIs of MSA patients and is found to be at higher levels in the CSF of MSA patients than in PD patients.

The MAPT gene has two main haplotypes, H1 and H2. Haplotypes are a group of genes that are passed down from a single parent and can be used to understand how traits and diseases are inherited. The H1 haplotype is more commonly associated with a higher risk of developing a neurodegenerative disease, whereas the H2 haplotype tends to decrease disease risk through a protective effect.

One study researched the haplotypes and sub-haplotypes of the MAPT gene through 213 Caucasian MSA patients of European descent and 1312 healthy control subjects [18]. Of the 213 MSA patients, 127 were pathologically confirmed, and 86 were clinically diagnosed. The pathologically confirmed cases were taken from a brain bank, and the clinically diagnosed cases, 78 probable MSA and eight possible MSA, were living individuals. DNA was extracted from white blood cells and brain tissue to assess six MAPT haplotype variants. The results showed a significantly increased risk for the H1J and H1x haplotypes and a protective effect for the H2 and H1E haplotypes. When evaluating the MSA patients, it was found that the H2 haplotype occurred less frequently in those with MSA-C, indicating greater disease risk. This implies that there are different risk associations for MSA-C and MSA-P concerning the MAPT gene. To corroborate these findings, the association between the MAPT gene and MSA should be further explored among other ethnic populations.

Testing for these genes is necessary to assess the risk of MSA and plan accordingly. For genetic testing to be used widely in identifying MSA, consistency in the methods and results of studies is essential. In many studies, DNA was obtained through blood and brain tissue, indicating that future studies would benefit from DNA extraction from these areas. There was more variation in whether the DNA was taken while the individuals were living or postmortem, as well as the stage and type of MSA. The studies involved MSA patients of possible MSA, probable MSA, pathologically proven MSA, clinically diagnosed MSA, etc. Taking into account whether the MSA was of the cerebellar type or Parkinsonian also affects whether or not specific genes may be present. Additionally, ethnicity plays a significant role in the presence of specific genes; studies of different ethnic populations revealed differences in findings of whether specific genes were associated with MSA or not. Additionally, these studies examined various mutations

and variants of the genes. In order to fully evaluate the extent to which these genes are present across various ethnic populations, consistent variants should be studied.

Conclusion:

An accurate clinical diagnosis of MSA is necessary for early intervention and appropriate disease management. The overlapping symptoms between MSA and other neurodegenerative disorders, especially PD, contribute to the complications and delay in diagnosis. In this paper, I reviewed the different methods that have been used in an attempt to diagnose MSA and distinguish it from other disorders accurately. The four levels of diagnostic certainty, possible prodromal MSA, clinically established MSA, clinically probable MSA, and neuropathologically established MSA, allow clinicians to assess disease severity through the duration of the disease and symptoms present. MRIs of various brain structures have been shown to be effective in distinguishing MSA, as there is greater atrophy of brain structures in MSA patients than in PD patients. Voxel-based morphometry studies show significant loss of gray and white matter in the cerebellum, brainstem, and middle cerebellar peduncle, as well as much greater cortical and subcortical atrophy in MSA patients than in PD patients. Diffusor tensor imaging revealed that MSA patients had increased mean diffusivity values and decreased fractional anisotropy values compared to PD patients and healthy control subjects, indicating loss of brain structure and tissue damage. Studying larger cohorts to ensure these findings are widespread, and increasing accessibility of these advanced techniques, would benefit MSA diagnosis. Of the imaging biomarkers, the hot cross bun sign has shown to be more promising than the putaminal slit sign, with higher specificity and sensitivity rates.

Various biomarkers have been studied regarding MSA, the most significant being the α-synuclein protein. CSF and red blood cell samples of MSA patients displayed greater α-synuclein aggregation than those of PD patients. Of the three primary forms of α-synuclein, t-α-syn, p-syn, and o-α-syn, p-syn seems to be the most reliable in diagnosing MSA and is most closely associated with disease progression. Metabolite concentrations of NAA and MI were measured in the pons and medulla of MSA-C patients to determine disease severity. Results showed that the levels of MI were much greater, and the levels of NAA much less, in MSA-C patients than in healthy controls.

The SNCA, CoQ2 (which codes for CoQ10), LRRK2, and MAPT genes have been found to have a possible association with MSA, allowing for a genetic method of identifying individuals at increased risk. However, there have been contradictions in the current research on the utility of screening for specific genes. Some studies indicate genetic associations with MSA were present [12, 13, 16-18, 25, 26]. Other studies reveal that those genetic associations are limited by ethnic populations, as results did not display significance across all ethnicities, namely within Asian populations [22, 23, 27, 28]. It is key to note that several contradictory studies screened for different variants of the genes, which influenced the outcomes. These disputed findings complicate genetic screening applications in diagnosing MSA and require further research.

Many studies have focused solely on differentiating MSA patients from healthy control subjects to identify signs of the disease. While these findings help give rise to the fact that a disease is present, most are the same symptoms shared with other neurodegenerative diseases. The key is in researching the severity of these symptoms; in most studies, it has been found that MSA patients tend to present with more severe levels of the same symptoms that PD patients have. For example, atrophy in the cerebellum occurs in both MSA and PD but occurs at

greater levels in MSA patients, and α-synuclein aggregates are far more prominent in the CSF of MSA patients than that of PD patients.

Reliable methods of diagnosing MSA from PD are essential because each disease progresses and is managed differently. MSA progresses at a greater rate than PD, as autonomic dysfunction can develop within a year, and symptoms tend to manifest earlier and more severely [21]. This also affects life expectancy: after symptoms begin, individuals with MSA have about six to nine years left, depending on severity, and those with PD have about fourteen to sixteen years left. Additionally, treatment options and symptom management are different. For example, those with PD often respond to a treatment called levodopa, used to treat bradykinesia, while most MSA patients have poor levodopa responsiveness [4, 9]. Effectively managing MSA disease progression, to help individuals who are suffering, requires an accurate and distinct diagnosis, only possible through the improvement of current resources and methods.

References:

1. Overk, C., Rockenstein, E., Valera, E., Stefanova, N., Wenning, G., & Masliah, E. (2018). Multiple system atrophy: experimental models and reality. *Acta neuropathologica*, *135*(1), 33–47. https://doi.org/10.1007/s00401-017-1772-0
2. Jellinger K. A. (2022). Heterogeneity of Multiple System Atrophy: An Update. *Biomedicines*, *10*(3), 599. https://doi.org/10.3390/biomedicines10030599
3. Tseng, F. S., Foo, J. Q. X., Mai, A. S., & Tan, E. K. (2023). The genetic basis of multiple system atrophy. *Journal of translational medicine*, *21*(1), 104. https://doi.org/10.1186/s12967-023-03905-1
4. Wenning, G. K., Stankovic, I., Vignatelli, L., Fanciulli, A., Calandra-Buonaura, G., Seppi, K., Palma, J. A., Meissner, W. G., Krismer, F., Berg, D., Cortelli, P., Freeman, R., Halliday, G., Höglinger, G., Lang, A., Ling, H., Litvan, I., Low, P., Miki, Y., Panicker, J., … Kaufmann, H. (2022). The Movement Disorder Society Criteria for the Diagnosis of Multiple System Atrophy. *Movement disorders : official journal of the Movement Disorder Society*, *37*(6), 1131–1148. https://doi.org/10.1002/mds.29005
5. Shahnawaz, M., Mukherjee, A., Pritzkow, S., Mendez, N., Rabadia, P., Liu, X., Hu, B., Schmeichel, A., Singer, W., Wu, G., Tsai, A. L., Shirani, H., Nilsson, K. P. R., Low, P. A., & Soto, C. (2020). Discriminating α-synuclein strains in Parkinson's disease and multiple system atrophy. *Nature*, *578*(7794), 273–277. https://doi.org/10.1038/s41586-020-1984-7

6. Krismer, F., Beliveau, V., Seppi, K., Mueller, C., Goebel, G., Gizewski, E. R., Wenning, G. K., Poewe, W., & Scherfler, C. (2021). Automated Analysis of Diffusion-Weighted Magnetic Resonance Imaging for the Differential Diagnosis of Multiple System Atrophy from Parkinson's Disease. *Movement disorders : official journal of the Movement Disorder Society*, *36*(1), 241–245. https://doi.org/10.1002/mds.28281
7. Specht K, Minnerop M, Abele M, Reul J, Wüllner U, Klockgether T. In Vivo Voxel-Based Morphometry in Multiple System Atrophy of the Cerebellar Type. *Arch Neurol.* 2003;60(10):1431–1435. doi:10.1001/archneur.60.10.1431
8. Krismer, F., Seppi, K., Göbel, G., Steiger, R., Zucal, I., Boesch, S., Gizewski, E. R., Wenning, G. K., Poewe, W., & Scherfler, C. (2019). Morphometric MRI profiles of multiple system atrophy variants and implications for differential diagnosis. *Movement disorders : official journal of the Movement Disorder Society*, *34*(7), 1041–1048. https://doi.org/10.1002/mds.27669
9. Osaki, Y., Wenning, G. K., Daniel, S. E., Hughes, A., Lees, A. J., Mathias, C. J., & Quinn, N. (2002). Do published criteria improve clinical diagnostic accuracy in multiple system atrophy?. *Neurology*, *59*(10), 1486–1491. https://doi.org/10.1212/01.wnl.0000028690.15001.00
10. Takado, Y., Igarashi, H., Terajima, K., Shimohata, T., Ozawa, T., Okamoto, K., Nishizawa, M., & Nakada, T. (2011). Brainstem metabolites in multiple system atrophy of cerebellar type: 3.0-T magnetic resonance spectroscopy study. *Movement disorders : official journal of the Movement Disorder Society*, *26*(7), 1297–1302. https://doi.org/10.1002/mds.23550
11. Wan, L., Zhu, S., Chen, Z., Qiu, R., Tang, B., & Jiang, H. (2023). Multidimensional biomarkers for multiple system atrophy: an update and future directions. *Translational neurodegeneration*, *12*(1), 38. https://doi.org/10.1186/s40035-023-00370-0
12. Monzio Compagnoni, G., Kleiner, G., Bordoni, A., Fortunato, F., Ronchi, D., Salani, S., Guida, M., Corti, C., Pichler, I., Bergamini, C., Fato, R., Pellecchia, M. T., Vallelunga, A., Del Sorbo, F., Elia, A., Reale, C., Garavaglia, B., Mora, G., Albanese, A., . . . Di Fonzo, A. (2018). Mitochondrial dysfunction in fibroblasts of Multiple System Atrophy. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1864*(12), 3588-3597. https://doi.org/10.1016/j.bbadis.2018.09.018
13. Al-Chalabi, A., Dürr, A., Wood, N. W., Parkinson, M. H., Camuzat, A., Hulot, J. S., Morrison, K. E., Renton, A., Sussmuth, S. D., Landwehrmeyer, B. G., Ludolph, A., Agid, Y., Brice, A., Leigh, P. N., Bensimon, G., & NNIPPS Genetic Study Group (2009). Genetic variants of the alpha-synuclein gene SNCA are associated with multiple system atrophy. *PloS one*, *4*(9), e7114. https://doi.org/10.1371/journal.pone.0007114
14. Butler, B., Sambo, D., & Khoshbouei, H. (2017). Alpha-synuclein modulates dopamine neurotransmission. *Journal of chemical neuroanatomy*, *83-84*, 41–49. https://doi.org/10.1016/j.jchemneu.2016.06.001
15. Tsika, E., & Moore, D. J. (2012). Mechanisms of LRRK2-mediated neurodegeneration. *Current neurology and neuroscience reports*, *12*(3), 251–260. https://doi.org/10.1007/s11910-012-0265-8
16. Riboldi, G. M., Palma, J. A., Cortes, E., Iida, M. A., Sikder, T., Henderson, B., Raj, T., Walker, R. H., Crary, J. F., Kaufmann, H., & Frucht, S. (2019). Early-onset pathologically proven multiple system atrophy with LRRK2 G2019S mutation. *Movement disorders : official journal of the Movement Disorder Society*, *34*(7), 1080–1082. https://doi.org/10.1002/mds.27710

17. Carrer, T., Bonato, G., Sandre, M., Emmi, A., Campagnolo, M., Musso, G., Carecchio, M., Parchi, P., & Antonini, A. (2024). Rapidly progressive multiple system atrophy in a patient carrying LRRK2 G2019S mutation. *Neurological sciences : official journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology*, *45*(1), 309–313. https://doi.org/10.1007/s10072-023-07056-5
18. Labbé, C., Heckman, M. G., Lorenzo-Betancor, O., Murray, M. E., Ogaki, K., Soto-Ortolaza, A. I., Walton, R. L., Fujioka, S., Koga, S., Uitti, R. J., van Gerpen, J. A., Petersen, R. C., Graff-Radford, N. R., Younkin, S. G., Boeve, B. F., Cheshire, W. P., Jr, Low, P. A., Sandroni, P., Coon, E. A., Singer, W., … Ross, O. A. (2016). MAPT haplotype diversity in multiple system atrophy. *Parkinsonism & related disorders*, *30*, 40–45. https://doi.org/10.1016/j.parkreldis.2016.06.010
19. Brenneis, C., Seppi, K., Schocke, M. F., Müller, J., Luginger, E., Bösch, S., Löscher, W. N., Büchel, C., Poewe, W., & Wenning, G. K. (2003). Voxel-based morphometry detects cortical atrophy in the Parkinson variant of multiple system atrophy. *Movement disorders : official journal of the Movement Disorder Society*, *18*(10), 1132–1138. https://doi.org/10.1002/mds.10502
20. Rulseh, A. M., Keller, J., Rusz, J., Syka, M., Brozova, H., Rusina, R., Havrankova, P., Zarubova, K., Malikova, H., Jech, R., & Vymazal, J. (2016). Diffusion tensor imaging in the characterization of multiple system atrophy. *Neuropsychiatric disease and treatment*, *12*, 2181–2187. https://doi.org/10.2147/NDT.S109094
21. Burns, M. R., & McFarland, N. R. (2020). Current Management and Emerging Therapies in Multiple System Atrophy. *Neurotherapeutics*, *17*(4), 1582–1602. https://doi.org/10.1007/s13311-020-00890-x
22. Katzeff, J. S., Phan, K., Purushothuman, S., Halliday, G. M., & Kim, W. S. (2019). Cross-examining candidate genes implicated in multiple system atrophy. *Acta Neuropathologica Communications*, *7*(1). https://doi.org/10.1186/s40478-019-0769-4
23. Sailer, A., Scholz, S. W., Nalls, M. A., Schulte, C., Federoff, M., Price, T. R., Lees, A., Ross, O. A., Dickson, D. W., Mok, K., Mencacci, N. E., Schottlaender, L., Chelban, V., Ling, H., O'Sullivan, S. S., Wood, N. W., Traynor, B. J., Ferrucci, L., Federoff, H. J., . . . Cortelli, P. (2016). A genome-wide association study in multiple system atrophy. *Neurology*, *87*(15), 1591–1598. https://doi.org/10.1212/wnl.0000000000003221
24. Sun, Z., Xiang, X., Tang, B., Chen, Z., Peng, H., Xia, K., & Jiang, H. (2015). SNP rs11931074 of the SNCA gene may not be associated with multiple system atrophy in Chinese population. *The International journal of neuroscience*, *125*(8), 612–615. https://doi.org/10.3109/00207454.2014.990013
25. Multiple-System Atrophy Research Collaboration (2013). Mutations in COQ2 in familial and sporadic multiple-system atrophy. *The New England journal of medicine*, *369*(3), 233–244. https://doi.org/10.1056/NEJMoa1212115
26. Sun, Z., Ohta, Y., Yamashita, T., Sato, K., Takemoto, M., Hishikawa, N., & Abe, K. (2016). New susceptible variant of COQ2 gene in Japanese patients with sporadic multiple system atrophy. *Neurology. Genetics*, *2*(2). https://doi.org/10.1212/nxg.0000000000000054
27. Yuan, X., Chen, Y., Cao, B., Zhao, B., Wei, Q., Guo, X., Yang, Y., Yuan, L., & Shang, H. (2015). An association analysis of the R1628P and G2385R polymorphisms of the LRRK2 gene in multiple system atrophy in a Chinese population. *Parkinsonism & related disorders*, *21*(2), 147–149. https://doi.org/10.1016/j.parkreldis.2014.11.022

28. Chen, Y. P., Zhao, B., Cao, B., Song, W., Guo, X., Wei, Q. Q., Yang, Y., Yuan, L. X., & Shang, H. F. (2015). Mutation scanning of the COQ2 gene in ethnic Chinese patients with multiple-system atrophy. *Neurobiology of Aging*, *36*(2), 1222.e7-1222.e11. https://doi.org/10.1016/j.neurobiolaging.2014.09.010
29. Soma, H., Yabe, I., Takei, A., Fujiki, N., Yanagihara, T., & Sasaki, H. (2006). Heredity in multiple system atrophy. *Journal of the neurological sciences*, *240*(1-2), 107–110. https://doi.org/10.1016/j.jns.2005.09.003
30. Hara, K., Momose, Y., Tokiguchi, S., Shimohata, M., Terajima, K., Onodera, O., Kakita, A., Yamada, M., Takahashi, H., Hirasawa, M., Mizuno, Y., Ogata, K., Goto, J., Kanazawa, I., Nishizawa, M., & Tsuji, S. (2007). Multiplex families with multiple system atrophy. *Archives of neurology*, *64*(4), 545–551. https://doi.org/10.1001/archneur.64.4.545
31. Li, X. Y., Yang, W., Li, X., Li, X. R., Li, W., Song, Q., Sun, L., Lin, F., Chen, Z., Wang, C., & Yu, S. (2020). Phosphorylated Alpha-Synuclein in Red Blood Cells as a Potential Diagnostic Biomarker for Multiple System Atrophy: A Pilot Study. *Parkinson's disease*, *2020*, 8740419. https://doi.org/10.1155/2020/8740419
32. Abdul-Rahman, T., Herrera-Calderón, R. E., Ahluwalia, A., Wireko, A. A., Ferreira, T., Tan, J. K., Wolfson, M., Ghosh, S., Horbas, V., Garg, V., Perveen, A., Papadakis, M., Ashraf, G. M., & Alexiou, A. (2024). The potential of phosphorylated α-synuclein as a biomarker for the diagnosis and monitoring of multiple system atrophy. *CNS Neuroscience & Therapeutics*, *30*(4). https://doi.org/10.1111/cns.14678
33. Foulds, P. G., Yokota, O., Thurston, A., Davidson, Y., Ahmed, Z., Holton, J., Thompson, J. C., Akiyama, H., Arai, T., Hasegawa, M., Gerhard, A., Allsop, D., & Mann, D. M. (2012). Post mortem cerebrospinal fluid α-synuclein levels are raised in multiple system atrophy and distinguish this from the other α-synucleinopathies, Parkinson's disease and Dementia with Lewy bodies. *Neurobiology of disease*, *45*(1), 188–195. https://doi.org/10.1016/j.nbd.2011.08.003
34. Sekiya, H., Kowa, H., Koga, H., Takata, M., Satake, W., Futamura, N., Funakawa, I., Jinnai, K., Takahashi, M., Kondo, T., Ueno, Y., Kanagawa, M., Kobayashi, K., & Toda, T. (2019). Wide distribution of alpha-synuclein oligomers in multiple system atrophy brain detected by proximity ligation. *Acta Neuropathologica*, *137*(3), 455–466. https://doi.org/10.1007/s00401-019-01961-w
35. Doppler, K., Weis, J., Karl, K., Ebert, S., Ebentheuer, J., Trenkwalder, C., Klebe, S., Volkmann, J., & Sommer, C. (2015). Distinctive distribution of phospho-alpha-synuclein in dermal nerves in multiple system atrophy. *Movement Disorders*, *30*(12), 1688–1692. https://doi.org/10.1002/mds.26293
36. Donadio, V., Incensi, A., Rizzo, G., Westermark, G. T., Devigili, G., De Micco, R., Tessitore, A., Nyholm, D., Parisini, S., Nyman, D., Tedeschi, G., Eleopra, R., Ingelsson, M., & Liguori, R. (2023). Phosphorylated α-synuclein in skin Schwann cells: a new biomarker for multiple system atrophy. *Brain*, *146*(3), 1065–1074. https://doi.org/10.1093/brain/awac124