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Multiple system atrophy: the application of genetics in understanding etiology

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Abstract

Classically defined phenotypically by a triad of cerebellar ataxia, parkinsonism and autonomic dysfunction in conjunction with pyramidal signs, multiple system atrophy (MSA) is a rare and progressive neurodegenerative disease affecting an estimated 3-4 per every 100,000 individuals among adults 50-99 years of age. With a pathological hallmark of alpha-synuclein-immunoreactive glial cytoplasmic inclusions (GCIs; Papp-Lantos inclusions), MSA patients exhibit marked neurodegenerative changes in the striatonigral and/or olivopontocerebellar structures of the brain. As a member of the alpha-synucleinopathy family, which is defined by its well-demarcated alpha-synuclein-immunoreactive inclusions and aggregation, MSA's clinical presentation exhibits several overlapping features with other members including Parkinson's disease (PD) and dementia with Lewy bodies (DLB). Given the extensive fund of knowledge regarding the genetic etiology of PD revealed within the past several years, a genetic investigation of MSA is warranted. While a current genome wide association (GWA) study is underway for MSA to further clarify the role of associated genetic loci and single nucleotide polymorphisms (SNPs), several cases have presented solid preliminary evidence of a genetic etiology. Naturally, genes and variants manifesting known associations with PD (and other phenotypically similar neurodegenerative disorders), including *SNCA* and *MAPT*, have been comprehensively investigated in MSA patient cohorts. More recently variants in *COQ2* have been linked to MSA in the Japanese population although this finding awaits replication. Nonetheless, significant positive associations with subsequent independent replication studies have been scarce. With very limited information regarding genetic mutations or alterations in gene dosage as a cause of MSA, the search for novel risk genes, which may be in the form of common variants or rare variants, is the logical nexus for MSA research. We believe that the application of next generation genetic methods to MSA will provide valuable insight into the underlying causes of this disease, and will be central to the identification of etiologic based therapies.

Keywords

Genome wide association; linkage analysis; genetic risk; mutation

Introduction

As a rare progressive neurodegenerative disease with an estimated incidence of 3-4 per every 100,000 individuals among adults 50-99 years of age, multiple system atrophy (MSA) is classically defined clinically by a triad of cerebellar ataxia, parkinsonism and autonomic dysfunction in conjunction with pyramidal signs. [1–3] From onset, with an average age of 57, to mortality, MSA typically progresses over 7-9 years and affects both sexes equally.[2, 4] However, given our limited understanding of the genetics and biomarkers of MSA, definite diagnosis can only be verified upon autopsy.[2] With an estimated false positive clinical diagnostic rate of approximately 14%, MSA's clinical presentation is often not recognized until later stages of disease progression, with very limited clinical ability to intervene.[5, 6] Histologically, MSA uniquely exhibits alpha-synuclein-positive glial cytoplasmic inclusions (GCIs), which are required for definitive diagnosis.[7]

While genetic studies have been very few in number, currently classifying MSA as a sporadic disease, a few familial studies have revealed an underlying genetic component of MSA.[4, 8] Moreover, it has been suggested that specific polymorphisms of the *SNCA* gene have been associated with an elevated risk of MSA among Caucasian populations.[9] As an etiologically and clinically complex disorder, MSA has been divided into subtypes based on predominant clinical features.[7] Several studies have described significant population-specific variation among MSA patients regarding frequency of one subtype over another. This notion further supports a role of genetic risk factors in the development of MSA pathogenesis.[10, 11] While *in vitro*, *in vivo* and transgenic studies continue to elucidate molecular mechanisms driving MSA etiology and pathology, the genetic basis of this disease still requires extensive investigation. We describe here the state of the field in MSA, and argue that it is critical to apply state-of-the-art genetic approaches to MSA.[1] Ultimately, understanding the molecular pathogenesis of this disease is our best hope to design and test etiologic based interventions.

Clinical and Neuropathological Features of MSA

While autopsy is necessary for a diagnosis of MSA, clinical diagnosis is often sought at the time of initial presentation.[2] This is based upon a thorough clinical evaluation, revealing motor dysfunction (either parkinsonism or cerebellar), and/or autonomic dysfunction (excluding erectile dysfunction). It is hypothesized that subclinical neuropathological changes may occur years before patients become clinically symptomatic.[2]

As a member of the alpha-synucleinopathy family, defined by well-demarcated alpha-synuclein-immunoreactive inclusions and aggregation, MSA's clinical presentation exhibits several overlapping features with other members including Parkinson's disease and dementia with Lewy bodies (DLB).[1] Hence, it is not surprising that there is an increased frequency (13%) of parkinsonism in 1st and 2nd degree relatives of autopsy proven MSA cases.[12] However, a positive family history of Parkinson's disease is not a significant risk factor for the development of MSA, an observation that is perhaps complicated by the difficulty of clinically diagnosing MSA.[13]

While MSA predominately consists of GCIs containing alpha-synuclein aggregates, it is important to mention that other protein aggregates, including hyperphosphorylated tau, can also be found. [14] Interestingly, MSA also exhibits extensive clinical overlap with members of the tauopathy family, including progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD).[15] In a similar fashion to MSA with fellow alpha-synucleinopathies like Parkinson's disease, autopsy confirmed cases of MSA, PSP and CBD, all of which are considered "atypical parkinsonisms," often present with phenotypes distinct from their "classical ones"; hence, MSA can present with a spectrum of clinical phenotypes (i.e. vertical gaze palsy), typically associated with tauopathies.[15, 16] To address this uncertainty, studies have scrutinized cases of atypical parkinsonisms to establish well-defined criteria to increase diagnostic accuracy in a clinical setting.[15, 16]

In addition to clinical features of alpha-synucleinopathies and tauopathies, MSA phenotypes can also overlap with subtypes of spinocerebellar ataxias (SCAs) and other familial ataxias. [17, 18] While most SCAs are negative for alpha-synuclein upon immunohistochemical staining, a few subtypes, such as *SCA3*, can exhibit glial alpha-synuclein-positive inclusions.[17, 18] Notably, some cases of *SCA3* can manifest levodopa-responsive parkinsonism, pyramidal tract dysfunction and even some dysautonomia, all of which are highly characteristic features of MSA; further, there may exist substantial clinical overlap between MSA and other genetic forms of SCA, including *SCA2*, *SCA6*, *SCA8*, and *SCA17*. Among 302 clinically diagnosed MSA patients, 7.3% were found to be SCA positive, of which more than half were *SCA17* carriers [18–25]. When MSA is in the differential diagnosis, genetic testing for the spinocerebellar ataxias in such patients is recommended to essentially rule out a familial ataxia.[25, 26]

Based on pathological investigation of regions predominately affected and their corresponding phenotypes, MSA has been subdivided into two distinct subtypes: MSA-Cerebellar (C), MSA-parkinsonism (P), with frequency varying in a population-specific manner.[1, 26] Despite this well-defined classification system of MSA, current treatment options for patients with either subtype is far from optimal: not only is there no treatment to delay the progression of disease, but levodopa is considered the primary treatment for symptoms, which has a "modest and non-sustained effect." [28, 29] While approximately 30% of MSA patients demonstrate an initial response to levodopa therapy, this response does not persist although patients often find it hard to stop this drug.[30]

As an oligodendroglialopathy with the pathological hallmark of widespread alpha-synuclein-immunoreactive GCIs (Papp-Lantos inclusions), MSA patients exhibit marked neurodegenerative changes in the striatonigral and/or olivopontocerebellar structures of the brain.[7] There is extensive variation in the degree of degeneration, depicted by a broad spectrum of myelin pallor, gliosis and neuronal loss; nonetheless, such features are key neuropathological manifestations of all MSA subtypes.[1] On a gross level, differences in size and pallor of affected regions can be particularly useful in differentiating MSA from CBD and PSP.[1] Furthermore, a handful of case reports have noted the coexistence of tau and alpha-synuclein inclusion bodies within autopsies of a single individual, suggesting a shared pathological mechanism, potentially through disruption of cytoskeletons and dislocation and aggregation of various proteins.[2, 31]

Corresponding with the clinical subtypes of MSA, gross pathological descriptions of cerebellar and parkinsonian subtypes parallel those same regions or systems predominantly affected by MSA pathology.[1] In cases of MSA-C, the olivopontocerebellar pathway is the primary focus, grossly presenting with a decreased cerebellar size, greatly reduced pons size, blurring of the inferior olive and extensive pallor of white matter within the cerebellum.[1] In contrast, MSA-P targets the striatonigral pathway. This leads to pallor of the substantia nigra and locus coeruleus, extensive darkening and atrophy of the putamen, yet grossly normal brainstem and cerebellar regions.[1]

Neuropathology: Molecular biology

Alpha-synuclein is not highly expressed in normal oligodendrocytes. Hence, upon studying the molecular biology of MSA, a critical question arises: how does alpha-synuclein ultimately end up in oligodendrocytes, despite its apparently neuronal site of origin? To elucidate a potential mechanism of redistribution, researchers have tested the hypothesis that oligodendrocyte uptake of alpha-synuclein is derived from neuronal secretion.[32] Analysis illustrated that in the presence of elevated alpha-synuclein levels, either in the form of soluble oligomers or intracellular alpha-synuclein inclusions in neurons, neuronal secretion is enhanced within rat brains.[32] Specifically, it was demonstrated that rat oligodendroglial cells *in vitro* internalized alpha-synuclein from neuronal secretions in a time, concentration and clathrin-dependent fashion.[32]

To further understand the redistribution process, Rockenstein and colleagues created transgenic mice models and studied heterozygous progeny. Among the parental mice, one expressed alpha-synuclein under an oligodendroglial-specific myelin-basic promoter and the other parental mouse expressed alpha-synuclein under a neuronal platelet derived growth factor promoter.[33] Upon studying compound transgenic mice progeny, a “robust redistribution” of alpha-synuclein was observed.[33] While the exact mechanism of action is unknown, it is hypothesized that a direct “translocation” through the extracellular space occurred via cell-cell interactions, moving alpha-synuclein from neurons to neighboring oligodendrocytes.[33] An oligodendroglial histological prototype, visualized in the basal ganglia and neocortex, was present in both the compound transgenic progeny and the parent expressing the oligodendroglial specific myelin basic promoter. Further assessment of the progeny revealed behavioral, pathological and chemical changes all consistent with the MSA-P phenotype. Notably, this was depicted in the progeny mice by physical motor deficits, substantial neuropathology in the basal ganglia, and decreased levels of immunoreactive tyrosine hydroxylase.[33] Collectively, these data suggest a predilection for alpha-synuclein accumulation in oligodendroglia relative to the neurons in regions of the brain susceptible to MSA, corresponding with classic pathophysiological changes seen in the disease.[33]

It has been suggested that specific MSA clinical subtypes, disease severity and duration of disease are all associated with the quantitative distribution and density of GCIs in MSA cases.[1] While GCIs represent the pathological hallmark of MSA, the abnormal accumulation of alpha-synuclein has also been identified within neuronal cytoplasmic inclusions (NCIs), neuronal nuclei inclusions (NNIs), and within neurites of a minority of

MSA affected brains. While these findings have not been the primary focus of MSA molecular research, the potential role of NCIs, NNIs and neurites in the pathological process of MSA warrants further investigation.[1, 34]

It has been suggested that the formation of GCI bodies is directly related to the degradation of oligodendrocytes. Midkine, a neurotrophic factor, is expressed by GCI-bearing cells in the brains of MSA patients, while absent in all oligodendrocytes, neurons and astrocytes of normal control brains.[35] Midkine, a novel heparin-binding protein, is known to enhance neuronal growth and survival among embryonic neuronal cells and induce mitogenesis within neuroectoderm-derived cells. Given midkine's selective presence in the GCIs of MSA brains, it has been suggested to function as a repair mechanism in response to oligodendrocyte and myelin degradation.[35–38]

Investigation of the extensive myelin pallor has been approached through a variety of immunohistochemical studies. One study designed antibodies to myelin basic protein (MBP), reporting that levels of MBP were substantially decreased in the white matter of the olivopontocerebellar and striatonigral systems of MSA brains.[1] Further, it has been shown that microglia are extremely active in MSA brains and have been visualized performing phagocytosis of degenerating myelin.[39] This observation corresponds well to classic histopathological findings of MSA: activated microglia and reactive astrocytes.[31] Furthermore, it has been suggested that the severity of astrogliosis, observed as swollen and bright GFAP-positive astrocytes, correlates with the degree of neurodegeneration within the olivopontocerebellar and striatonigral systems of MSA brains.[31]

As a protein of great interest regarding MSA pathology, tubulin-polymerization-promoting protein (TPPP), also known as p25alpha, functions in the stabilization of microtubules and the differentiation of oligodendrocytes.[40] Further investigation has revealed a significant reduction in the total levels of MBP and a simultaneous increase in MBP degradation products in conjunction with a redistribution of TPPP.[41, 42] This redistribution of TPPP involves movement from its myelin origin into the cell soma of oligodendrocytes; consequently, in cells containing GCIs, the overall cell body size is increased, suggesting a correlation between TPPP migration and GCI formation.[1] By promoting the formation of alpha-synuclein oligomers via direct interaction with alpha-synuclein, TPPP accelerates the formation of GCIs and oligodendroglial cell death.[41] Based on these observations, it has been hypothesized that in the early stages of MSA pathogenesis, TPPP and MBP interact directly, inducing MBP degradation and potential “redistribution” of TPPP from myelin to oligodendrocyte cell soma. Once within oligodendrocytes, TPPP enhances alpha-synuclein aggregation, as visualized by immunohistochemical staining within the GCIs.[1]

Given TPPP's suggested role in MSA pathogenesis, further analysis has revealed that phosphorylation of alpha-synuclein at *Serine-129* enhances TPPP-mediated oligomerization.[41] Alpha-synuclein *Ser-129* phosphorylation is thought to induce the formation of an unfolded state, leading to increased aggregation of insoluble alpha-synuclein by TPPP.[43, 44]

As a consequence of the alpha-synuclein oligomerization, oligodendroglial cells are stimulated to undergo apoptosis; thus, the anti-apoptotic effects of several proteins may serve as potential therapeutic candidates. For example, the inhibition of a tubulin deacetylase, *SIRT2*, has been demonstrated to partially rescue alpha-synuclein containing oligodendroglial cells from programmed cell death.[41] Notably, sirtuins are known to play a role in neuronal and glial cell function, with *SIRT2* presumed to accelerate neurodegeneration.[41] Previous studies demonstrated that *SIRT2* inhibition in neuroglioma cell lines can prevent alpha-synuclein mediated toxicity and modify GCI formation. More recent work has demonstrated that under conditions of increased levels of insoluble alpha-synuclein aggregates, administration of a *SIRT2* inhibitor rescues oligodendroglia by preventing apoptosis.[41, 45] While the specific mechanism has yet to be elucidated, it has been suggested that *SIRT2* inhibition may induce hyperacetylation of microtubules, facilitating microtubule stability. Consequently, a more robust microtubule network *may* enhance microtubule-dependent transport of insoluble alpha-synuclein aggregations out of oligodendrocyte nuclei and into the perinuclear space, forming “juxtannuclear large inclusions.” As a result, this may limit the burden of cytotoxicity and potentially rescue oligodendroglia from cell death via microtubule-mediated removal of intranuclear toxic alpha-synuclein aggregations.[41]

Understanding Etiology

As with many diseases, a sensible route to understanding MSA is to attempt to identify and understand the events that increase risk for MSA, and in doing so provide tools with which to model and study the pathogenic process. Also as with similar diseases, there are two areas of risk factor investigation that immediately come to mind: those of environmental and genetic origin.

Relatives of MSA patients have had significantly more clinical symptoms than did controls, and this, along with other work has been used as an argument to suggest a genetic or shared lifestyle etiology component for MSA. It is also noteworthy that the prevalence of MSA subtypes varies considerably among distinct ethnic groups: in the British population, MSA-P accounts for an estimated 34% of MSA cases, with MSA-C attributing only 17% and the remaining 49% considered a hybrid of equally severe cerebellar and parkinsonism pathology. In contrast, MSA-P in the Japanese population is considered much more rare (17%), while MSA-C is the predominant single subtype, accounting for 40% of all MSA cases, and 42% representing the remaining hybrids. [46] Again, while this cannot be attributed to a genetic, environmental, or lifestyle influence, such differences do suggest that there are likely discrete factors that influence this disease.

A preliminary study of MSA and occupational risk factors suggested that MSA patients had significantly more exposures to a variety of hazardous substances including plastic monomers and additives, organic solvents, pesticides and metal dusts and fumes.[47] As with PD occupational farming has been suggested to be associated as a risk factor for MSA, and again, as with PD a history of smoking is associated with a decreased risk for MSA.[13, 48] The role of cholesterol in MSA has also been investigated, perhaps in part because cholesterol has been suggested to interact with alpha-synuclein *in vitro*, potentially altering

its conformation and degree of aggregation. [49] One study looked at the association between the risk of MSA and serum cholesterol levels, revealing that reduced levels of high density lipoprotein cholesterol (HDL-C) and total cholesterol may be associated with an elevated risk of developing MSA, but not the duration or severity of disease.[50]

The concept that many disorders are complex diseases centers on the hypothesis that diseases can occur as a result of a complex interaction of genetic, environmental, and lifestyle factors. This concept has been a popular idea applied to many other late onset neurodegenerative diseases, and it is certainly true that evidence suggests in diseases such as AD and PD multiple genetic risk factors exist that individually exert small and moderate effect. It is likely, but unknown, that MSA will possess a similar etiologic architecture to these disorders, and therefore we should not be looking for either an environmental or genetic cause, but rather accept that the two are likely to coexist as contributors to the disease process.

Unfortunately, the relative rarity of MSA, and the difficulty in performing prospective epidemiological studies means that investigation of a potential role for environmental or lifestyle factors in this disease is relatively sparse, and to date no equivocal risk factor has been found. Further, the environment is an intrinsically difficult thing to study. The environment is almost infinite, particularly when taken in to consideration that different exposures are likely to have different effects depending on dosage, duration, and timing of exposure. Conversely, while the genome is certainly large and complex, genetics as a field has a good grasp of how to use modern methods to understand the genetic basis of complex diseases. We propose therefore that while relatively little has been performed within the context of MSA genetics, now is the time that we should consider tackling this difficult problem.

What is Known About The Genetics of MSA

The description of several MSA families suggest preliminary evidence of a genetic etiology at least in some families.[4, 8] While these are generally rare, understanding the genetic causes of rare familial forms of disease has provided successful insight into several common neurodegenerative diseases.

There has been one German family described with a rare Mendelian autosomal dominant form of definite MSA, one British family with definite MSA among two first cousins, and four different multiplex Japanese families reported with an autosomal recessive inheritance pattern, only one of them with pathologically confirmed MSA.[4, 8, 51] In all of the Japanese families, there were two siblings affected with MSA. Given the rare estimated frequency (3-4 per 100,000 among adults 50-99 years of age) of MSA among the general population, the probability of occurrence in two siblings within the same family, by chance, is approximately 6×10^{-5} , making this unlikely (although not impossible) to occur by chance. Further, studies have demonstrated that in the relatives of MSA patients, there is an increased prevalence of other neurodegenerative diseases.[52]

Based on a small number of family based studies, kindreds do exist with what appears to be MSA, inherited in an apparent autosomal dominant or recessive inheritance manner.[4, 8] While these families are likely to be key in our understanding of the genetic basis of this disorder, to date family-based gene discovery efforts have been few, and thus far not completely successful in MSA.

COQ2 mutations in MSA

Perhaps the most progress has been made in this regard with the publication of a recent finding by Tsuji et. al, which suggested that rare variants of *COQ2*, the gene encoding coenzyme Q2 4-hydroxybenzoate polyprenyltransferase, play a role in both familial and sporadic MSA. Specifically, members of a consanguineous Japanese family with MSA-P were reported to be homozygous for *COQ2* variants, p.M78V and p.V343A.[53] The latter variant, p.V343A, which is a common variant within the Japanese population, demonstrated a significant association with sporadic MSA cases in comparison to controls.[53] Finally, a yeast complementation assay was used to demonstrate that p.V343A variants, as well as other unique variants in *COQ2*, are correlated with dysfunction of *COQ2*. [53] As an antioxidant that prevents free radical damage and mitochondrial oxidative stress, *COQ2* is an interesting candidate gene to study, as it directly parallels our current hypothesis of neuropathology: neuroinflammation induced neurotoxicity and resulting neurodegeneration. [53]

In response to this interesting work, independent replication has been attempted by several other groups, all with very limited success. Primarily, other groups have clarified that Tsuji et. al used the shortest isoform [53-55], encoding the smallest protein of *COQ2*, which consequently affects the location of the noted homozygous mutations and does not cover a common nonsense variant at the initial sequence of the first exon.[53-55] Upon sequencing *COQ2* in a large Korean cohort, the p.V343A mutation, now designated by its location in the largest isoform, p.V393A, did not manifest any association with MSA cases. [54] Further, investigation of an extensive European cohort of clinically diagnosed MSA patients by candidate variant investigation found this same mutation in one case and one control, thus rejecting a potential association between this homozygous variant (p.V393A) and MSA. [55] Finally, the largest cohort of European pathologically confirmed MSA cases was analyzed by gene sequencing by Schottlaender et. al, who found unique *COQ2* variants with a higher frequency in controls than cases, and the absence of p.V393A in both cases and controls. [56] As a result, Tsuji et al have acknowledged these more recent findings and emphasize a more cautious approach to interpretation of their original results. [53] Recently, it has been hypothesized that variants in *COQ2*, which can inhibit normal gene function of coenzyme Q10, may prevent oligodendrocyte's ability to maintain lipid laden myelin sheath, resulting in increased oligodendrocyte apoptosis and increased risk of MSA. [57] While this is certainly interesting work, independent replication is required to firmly establish any etiological link of *COQ2* and MSA.

Genes encoding proteins involved in oxidative stress

It has been hypothesized that several genes that play a role in oxidative stress, inflammation and mitochondrial dysfunction may exhibit rare variants that increase genetic predisposition

towards the development of MSA.[26] In particular, studies have suggested positive association between cytokine gene polymorphisms and MSA genetic vulnerability.⁶⁸ As cytokines are key players in immunity and inflammation, such findings are consistent with MSA as a neuroinflammatory process. In one study, eight distinct candidate genes involved in oxidative stress were investigated. The data suggested that *SLC1A4*, *SQSTM1*, and *EIF4EBP1* demonstrated a significant association with MSA, though follow-up investigations are required for verification.[58] In addition to cytokines, several chemokines and inflammatory markers are produced upon microglial activation, inducing a neuroinflammatory response.[39] Specifically, variants found in *IL-1a*, *IL-1B*, *IL-8* and *ICAM-1* genes have all demonstrated an association with MSA. [59–62] Likewise, a polymorphic region within the tumor necrosis factor (TNF) gene, as well as a variant within alpha-1-antichymotrypsin gene, also manifested an association with MSA. [63, 64] Once again, these findings have not yet been convincingly replicated, and therefore should be interpreted with caution.

PRNP and MSA

Interestingly, Shibao et. al reported a case with a patient exhibiting both MSA and Creutzfeldt-Jakob disease (CJD). While these two diseases share some histopathological features, including the atypical abundance of alpha-synuclein proteins within the central nervous system, they had not previously been known to co-exist in a single individual. [65, 66] Normal prion protein exhibits resistance to oxidative stress, but develops increased susceptibility upon conversion to the infectious, pathological isoform. Given the overlapping histopathology of MSA and prion disease, Shibao et. al hypothesized that the abnormal prion protein may enhance sensitivity towards oxidative stress and consequently contribute to MSA pathogenesis.[65] While homozygosity of the p.M129V allele of prion protein (encoded by *PRNP*) is a known risk factor for CJD, the patient did not exhibit any mutations in *PRNP*, but the proband was homozygous MM for the p.M129V allele. To determine if an association exists between MSA and the p.M129V genotype, a case-control study was implemented. Results revealed no significant difference in the genotype frequencies between MSA cases and controls, but an increased prevalence of homozygosity (MM or VV) and younger onset of disease in MSA cases in comparison to PD cases.[65] While this is intriguing, the absence of abnormal prion proteins within GCIs of pathologically confirmed MSA patients is not trivial, casting doubt on the previous association.[67] Thus, in order to further elucidate the inflammatory etiology underlying MSA pathophysiology and a potential association with CJD, additional studies to seek out (new and confirm previous) inflammatory marker associations are vital. [26]

SNCA

The remarkable discoveries of gene mutations in *SNCA* encoding alpha-synuclein have provided critical insight into the genetic architecture, pathology, and etiopathogenesis of the most common synucleinopathy, PD.[68–70] While Lewy bodies are the hallmark neuropathological findings in Parkinson's disease, they can be found in approximately 10% of MSA cases. Likewise, mutation(s) of genes classically linked to PD, such as a p.G51D *SNCA* mutation, can also result in MSA pathology. For example, a recent study of a British patient with autosomal dominant young-onset Parkinson's disease possessing a p.G51D

SNCA mutation exhibited strikingly similar neuropathological and cellular features to a typical MSA case.[9] While this patient was levodopa responsive, the autopsy revealed a very high prevalence of GCI-like pathology within the cerebellar white matter, pontine base, and white matter underlying the motor cortex.[9] Furthermore, this case demonstrated positive immunoreactivity for alphaB-crystallin, a GCI-marker; hence, this provides additional support for a common pathogenic mechanism behind MSA and Parkinson's disease.[9] In addition to point mutations, whole gene duplications and triplications of *SNCA* can cause a progressive synucleiopathy. Specifically, the *SNCA* gene has been shown to be duplicated or even triplicated in forms of early onset Parkinson's disease, exhibiting a Mendelian form of inheritance.[68] Studying the neuropathology of affected family members harboring a *SNCA* triplication revealed the presence of abundant GCIs, characteristic of MSA histopathology.[68] Despite finding GCI-like inclusions in select cases of Parkinson's disease due to a *SNCA* triplication, *SNCA* sequencing, gene dosage, haplotype tagging and microsatellite studies of MSA have failed to disclose disease causing mutations.[69, 71–74] In addition, gene expression studies have failed to detect any changes in transcription of *SNCA* among confirmed MSA cases.[46, 75–77]

Although no coding mutations in *SNCA* have been found, a focused genotyping study of MSA demonstrated a significant association between particular SNPs within the *SNCA* locus and an increased risk of MSA among Caucasians.[6] Follow-up studies initially confirmed these SNP associations, with the most significant found in the MSA-C subtype. [78, 79] Located in the *SNCA* locus, two identified SNPs (rs3822066 and rs11931074), are presumed to be confined within a single haplotype block.[79] This block, extending from intron 4 to the 3' untranslated region (UTR) of the *SNCA* gene, is thought to be in strong linkage disequilibrium with the *SNCA* gene.[26] Moreover, these results have been found in a different cohort of pathologically confirmed MSA cases, providing further support of an association between MSA and this particular *SNCA* locus.[78] Interestingly, Parkinson's disease has exhibited a significant association for this very same haplotype block.[80, 81] While this suggests a common genetic etiology behind PD and MSA, investigations of an association between MSA cases and the risk variants located within this haplotype block have been elusive; indeed, Yun et al. observed an equal allelic frequency of Caucasian risk variants between MSA cases and controls among the Korean population. [26, 82] Such studies emphasize the need for independent replication across diverse populations, as the inter-population heterogeneity creates an additional layer of complexity to interpret the results of several association studies. In a similar fashion, intra-population heterogeneity has also demonstrated to be an important consideration: two snps in *SNCA*, rs2736990 and rs356220, which have demonstrated to be risk alleles for PD in a Chinese population, failed to show any association with either MSA or amyotrophic lateral sclerosis (ALS) in that same Chinese population. [83] Hence, by performing association studies among several potentially related yet clinically distinct neurodegenerative disorders within a single genetically homogenous population, intra-population heterogeneity may provide clues to the degree of overlap of pathological mechanisms underlying such disorders. Thus, while *SNCA* loci association studies remain intriguing, replication among and within distinct ethnic groups, in conjunction with whole-genome analysis, will be essential to confirm or reject these associations.

Other PD linked genes and risk for MSA

In addition to *SNCA*, several studies have investigated the prevalence of other known PD risk genes and variants among MSA cases. In the Ashkenazi Jewish population, an SNP (rs1572931) within a *RAS* oncogene family-like-1 (*RAB7L1*) promoter region has been shown to be protective against PD.[84, 85] Upon studying a Chinese population, rs1572931 demonstrated a protective effect for only late-onset PD patients. Nonetheless, there was no association detected, in either MAF or genotype frequency, with early-onset PD, MSA and ALS cases in the Chinese population. [85]

Several studies have also examined *MAPT*, encoding the protein tau, for variability that may impart risk for MSA. These studies have been inconsistent in their conclusions, with some reporting an association between the H1 haplotype of the *MAPT* locus with both MSA and PD,[80, 86] and others reporting a lack of significant associations between MSA and *MAPT* variants, confounding our current picture.[6, 72] Interestingly, a significant association between a particular *MAPT*H1-specific SNP (rs242557) and PSP has been noted, but lacks any association with PD; however, the H1/H2 ratio of two distinct SNPs (rs1052553, and rs62063857, respectively) manifests a strong association with PD. More recent GWAS in PD have shown an extremely robust association between *MAPT* variants and risk for PD. [80, 81, 87] Hence, such studies have demonstrated that unique sub-haplotypes within *MAPT* exhibit associations with both synucleinopathies and tauopathies, suggesting common, yet distinct etiologies.[87]

Mutations in *GBA*, encoding glucocerebrosidase, cause the autosomal recessive lysosomal storage disorder Gaucher's disease. Carrying a single *GBA* mutation, while not sufficient to cause Gaucher's disease, is a significant well established risk factor for PD, increasing the risk for this disease approximately 5 fold and the risk for DLB at a similar amount. [88] *GBA* variability has been investigated within MSA in order to determine whether this risk effect is generalizable to all synucleinopathies. [88, 89] While MSA and GBA-PD manifest several common clinical symptoms, screening for the PD associated *GBA* mutation among MSA patients has failed to reveal an association thus far. [89, 90]

Mutations in Leucine-rich kinase 2 gene (*LRRK2*), encoding dardarin, have been shown to account for about 3-10% of cases of familial Parkinson's disease and 1-8% of sporadic Parkinson's Disease cases.[70] Further, histopathological studies of brains expressing the *LRRK2* mutation also exhibited overlapping features of MSA neuropathology.[70] Despite these similarities, initial association studies between *LRRK2* mutations and MSA have all been negative.[91, 92] More recently, however, a collaborative study including two large series of neuropathologically confirmed MSA cases showed a significant association between *LRRK2* variants and MSA with a protective effect. [93] Again, as with many genetic studies in MSA, investigation of this gene in a larger cohort of MSA patients is warranted.

As the most common cause of autosomal-recessive early onset PD, mutations in Parkin and PTEN-induced putative kinase 1 (*PINK1*) have been analyzed among a pathologically confirmed MSA cohort.[94, 95] Results demonstrated the absence of pathogenic homozygous mutations in any MSA cases; while some manifested heterozygous variants,

this was not considered a statistically significant association.[95] Hence, the results suggest that despite shared clinical and pathological features, MSA and PD vary in at least some of their genetic origins.

Several other genes, including alcohol-dehydrogenase genes, *ADH1C* and *ADH7*, as well as ubiquitin C-terminal hydrolase-1, *UCHL-1*, have been suggested to manifest an association with PD.[96–98] After studying these genes in MSA cohorts, findings have revealed no association for *ADH7* and *UCHL-1* in MSA patients.[99, 100] Notably the association of variability at these two genes with PD still remains questionable.

C9orf72 hexanucleotide repeat expansion

Pathogenic expansion of the hexanucleotide repeat within *C9orf72* is the most common genetic cause of both amyotrophic lateral sclerosis and frontotemporal dementia.[101] Interestingly, a case study has recently demonstrated the coexistence of amyotrophic lateral sclerosis (ALS) and MSA in a single family.[101, 102] While pathological evaluation is awaiting confirmation of a diagnosis of definite MSA, the patient presented a hot cross bun sign on brain MRI. Further, she demonstrated ataxia, parkinsonism, autonomic dysfunction and rapid progression, which are all consistent with her diagnosis of possible MSA, while genetic testing of the spinocerebellar ataxias was negative. However, Schottlaender et. al and Scholz et. al were both unable to find this mutation among their respective MSA cohorts, suggesting that no association between *C9orf72* and MSA can be confirmed until MSA is proven upon autopsy. [103–105] Thus, such a case provides insight into a potentially overlapping genetic etiology between MSA and ALS, despite their distinct classical presentation of symptoms.

Other candidate genes

Several case reports have demonstrated an association between parkinsonism and Proximal myotonic myopathy (*PROMM*) or Myotonic dystrophy type 2 (*DM2*), which is a complex muscle disorder inherited in an autosomal dominant fashion.[106–108] Further, the *DM2* mutation exhibits an association with a levodopa unresponsive, atypical PD.[108, 109] Within the last couple of years, a case clinically diagnosed as probable MSA developed symptoms of muscle weakness and tested positive as a carrier of the *DM2* mutation.[110] Thus, while pathological confirmation of this case is necessary to confirm this association, this suggests a possible shared genetic origin of MSA and *DM2*.

Among the phenotypical triad of MSA includes autonomic dysfunction, which utilizes norepinephrine (NE) as the primary neurotransmitter to regulate normal physiological function within the autonomic nervous system (ANS). [7, 26] Since mutations in the dopamine-B-hydroxylase (*DBH*) gene result in a reduction of NE levels, it has been hypothesized that this may induce the ANS failure exhibited in MSA. However, when patients with MSA, pure autonomic failure, orthostatic intolerance, and controls were all tested for 7 distinct mutations within *DBH*, no one manifested any pathological variants. While this was a small study, involving only 39 individuals with MSA, it suggests that the NE deficiency due to *DBH* gene mutations are not a likely etiology of autonomic dysfunction in MSA. [111].

Other studies have investigated any potential association that may exist between MSA and the e4 allele of apolipoprotein E (*APOE*), given that e4 manifests a strong association as a risk factor for DLB and Alzheimer's disease (AD), although not with PD.[112] Given that DLB is in the alpha-synucleinopathy family, association studies have been conducted between *APOE* genotype and MSA. Results from these studies have been largely negative, suggesting that the e4 *APOE* genotype is not a risk allele for MSA. [112–114]

Genetic forms of Ataxia and MSA

In addition to investigating genes with known associations to PD and other alpha-synucleinopathies, other candidate genes, including several within the spinocerebellar ataxia (*SCA*) family, have been studied. One case involved a patient with a diagnosis of MSA-C harboring a *SCA1* triplet repeat expansion. [17] Neuropathological examination revealed pathology in several areas classically affected by MSA-C (cerebellum, brainstem), but also included GCIs, positive for both ubiquitin and tau. While this was indeed promising, other symptoms manifested by the patient did not resonate with typical MSA-C, such as the absence of pyramidal or extrapyramidal signs, despite cerebellar and autonomic dysfunction. [17]

In a family with familial olivopontocerebellar atrophy (OPCA), a son with clinical features resembling MSA-P manifested a CAG repeat expansion within the *SCA2* gene. Neuropathological examination displayed ubiquitin-positive but alpha-synuclein-negative GCIs, making MSA an implausible diagnosis. [26, 115]

One case study involved a patient with a late-onset phenotype consistent with MSA-C manifesting heterozygosity for a *SCA3* allelic expansion. Interestingly, autopsy of this case confirmed MSA through the presence of alpha-synuclein positive GCIs.[18] This is particularly noteworthy, as it suggests a common genetic etiology or shared pathological mechanism behind *SCA3* and MSA-C. Nonetheless, several more pathologically confirmed MSA cases, preferably familial, will be required to replicate these findings.[18]

Although patients with *SCA6* may present with levodopa-refractory parkinsonism, studies looking at a potential association between *SCA6* and MSA have been negative thus far. [21, 26]

In a case with neuropathology resembling MSA, including both ubiquitin and alpha-synuclein positive GCIs, genetic testing demonstrated that the patient exhibited unstable CTA/CTG repeats in *SCA8*. [116] Likewise, several patients harboring *SCA17* trinucleotide repeat expansions have manifested a phenotype characteristic of classic MSA.[24, 26] However, a follow-up study in a Japanese MSA cohort found that all 105 patients tested negative for the following *SCA* gene repeat expansions: 1, 2, 3, 6, 7, 8, 12, and 17.[63] Ozawa et. al has suggested that population variation of *SCA* genotypes should be taken into consideration, as *SCA1* and *SCA2* are more common in Caucasians, while *SCA3* and *SCA6* maintain a higher frequency within the Japanese population. [46] Thus, while this may be attributed to population heterogeneity, it may also suggest a lack of association between MSA and any types of *SCA*.

In addition to spinocerebellar ataxias, other forms of ataxia with clear genetic etiology, including Fragile X-associated ataxia syndrome (FXTAS) and Friedrich's ataxia (FA), may present sporadically; hence, studies have shown there is a 15-20% chance of a mutation among ataxia patients in the absence of a positive family history. [23, 26] Clinical features, including levodopa unresponsive parkinsonism, autonomic features (i.e. incontinence) and late-onset cerebellar ataxia, are shared among both FXTAS and MSA patients. [117] Further, delineated in about 40% of MSA-C cases, MRI studies illustrate a T2-weighted signal intensity increase in the middle cerebellar peduncles in both diseases. Neuropathological analysis of FXTAS brains reveals strictly intranuclear (vs. perinuclear) ubiquitin-positive inclusion bodies, negative for synuclein. [118–120] Despite some variation in histopathology, it has been hypothesized that a pre-mutation in the fragile X mental retardation gene (*FMR1*) may be a risk allele for developing MSA. [120] However, the findings among follow-up studies in Japanese and European cohorts have suggested that any association between *FMR1* and MSA is highly improbable. [117, 120, 121]

Copy number changes and MSA

Copy number variants (CNVs) are structural variants within the human genome; these strictly encompass deletion or multiplication of genomic segments that may or may not contain genes.[122] Often included within this class however, are copy neutral rearrangements, where a particular segment of genomic DNA is not lost or copied, but rather present in a different position, or orientation within the genome. [122]

As discussed above, copy number mutation at the *SNCA* locus is already linked to MSA through the presence of GCI pathology in carriers.[68] In part because of technologies that now make discovery and typing of CNVs possible, there has been increasing interest in the role such structural genomic alterations may play in the disease process. [122] Surprisingly, given that assessment of CNVs remains quite difficult and specialized, MSA has been studied in this regard, although it should be noted the studies performed thus far are modest in size. [123, 124] One study performed whole-genome CNV analysis in a 32-person Japanese MSA cohort, as well as a set of monozygotic twins discordant for MSA clinical diagnosis. [123] Analysis revealed copy number loss of the Src homology 2 domain containing transforming protein 2 (*SHC2*) among the single twin with MSA, as well as 20 of the other MSA patients, while not found in controls.[123] As CNVs are known to induce genomic instability and can lead to unequal crossing over or end-joining events during meiosis, the results suggest that this CNV-rich subtelomeric site may be susceptible to insertion, deletion or duplication events. [125] Further, CNVs in genes are known to have numerous potentially deleterious effects, including modified expression in a cis or trans fashion, and the formation of unstable mRNA and protein products, possibly causing pathophysiology.[126] Given that Shc proteins are known to play a role in neuronal cell development, acting as molecular switches for proliferation and differentiation, the potential for pathophysiology is not unlikely.[123]

Interestingly, the discordance among monozygotic twins suggests a form of somatic mosaicism to have occurred.[127] Thus, while there may be a genetic susceptibility towards developing MSA, certain environmental factors may be key towards turning on and off

genes, thereby modulating genetic expression and potentially inducing MSA pathophysiology. In a follow-up study, Ferguson et. al was unable to find CNVs in the *SHC2* gene among a non-Japanese MSA cohort. [124] Hence, while *SHC2* CNV analysis requires independent replication in a larger Japanese cohort and among diverse populations, the results from Sasaki et. al are promising for future studies.

While progress is being made in understanding the genetic basis of this disease, more needs to be done. This is particularly difficult in a disease such as MSA, not only because funding for these efforts is difficult to obtain, but also because this is a rare disease, and many of the state-of-the-art methods require large numbers to yield sufficient statistical power. However, the opportunities that exist for the genetic dissection of complex disease are markedly better than a decade ago, and it would seem critical that we attempt to push genetic progress in MSA.[1]

Proposed mechanisms of MSA pathogenesis

Although little is known about the genetic basis of MSA there has been some work centered on understanding the molecular pathogenesis of this disease. This has largely been derivative based on work ongoing in PD rather than based on unique molecular aspects of MSA.[2]

Role of Neurotoxicity and Oxidative Stress

As microglial activation is known to be associated with neuronal loss, the initiation of extensive microglial over-activation in olivopontocerebellar and striatonigral regions of the brain in MSA is intriguing.[2, 128] One such study demonstrated microglial transition into a state of over-activation upon exposure to environmental toxins and endogenous proteins. [129] This microglial excitability, specifically triggered by pattern recognition receptor transduction mechanisms, induces a release of reactive oxygen species (ROS), well-known culprits of facilitating neurotoxic states.[129]

In a transgenic mouse model, investigators induced alpha-synuclein overexpression (with a PLP promoter) in conjunction with exposure to toxin 3-Nitropropionic acid (3-NP). Histopathological analysis revealed GCI-like inclusions with a significant reduction of neurons in regions primarily targeted by MSA pathology: olivopontocerebellar and striatonigral systems.[128] Phenotypically, there was also a decline in motor and cerebellar function. Interestingly, increased levels of inducible nitric oxide synthase (iNOS), which plays a role in immunity and free radical propagation, was observed in the substantia nigra pars compacta.[128] Further, a direct correlation was observed between elevated iNOS levels with both the disappearance of striatonigral dopaminergic neurons and a rise in microglial activation, specifically in the substantia nigra pars compacta.[128]

These findings provide potential insights into our understanding of MSA pathogenesis. Principally, they suggest an increased susceptibility of this region to oxidative stress, which may serve as an initiation for neuroinflammation.[128] Building upon this notion, anti-neuroinflammatory agents have been tested in transgenic mice. While its anti-neuroinflammatory properties are somewhat elusive, long-term minocycline treatment was administered in the transgenic mice; As a result, microglial activation was inhibited in the

substantia nigra pars compacta, protecting dopaminergic neurons in this region.[128] While the mechanism of action is uncertain, potentially neuroprotective agents warrant further investigation, as it appears that oligodendroglial overexpression of alpha-synuclein in GCIs and oxidative stressors are obvious culprits in this devastating neurodegenerative disease.

As a constituent of the lipid component of the cell membrane, Docosahexaenoic acid (DHA) has been demonstrated to increase cell sensitivity to oxidative stress.[130] When increased levels of DHA are present within the cell membrane, heat shock protein expression increases, which is known to rise under conditions of oxidative stress. [130] With regard to MSA pathology, oligodendroglial cells with elevated DHA levels, expressing alpha-synuclein, are increasingly sensitive to oxidative stress. Further, it has been observed that this rise in oxidative stress sensitivity actually makes alpha-synuclein more insoluble, forming fibrillary inclusion bodies like those seen in MSA.[44] Such aggregate formation is simultaneously enhanced through an increase in phosphorylation of alpha-synuclein at serine-129, resonating with classic MSA pathology.[44]

Further study of the role of oxidative stress has investigated myeloperoxidase, a critical enzyme that plays a role in phagocytosis associated cell production of ROS.[131] As it exists in both human and mouse brains, and myeloperoxidase-containing macrophages and microglia have been observed in the CNS among other neurodegenerative diseases including PD, myeloperoxidase manipulation serves as a valuable enzymatic tool to elucidate the role of neuroinflammation and oxidative stress in MSA.[131] Several experiments have demonstrated that neuroinflammation is a “prominent pathological finding” in MSA, which is a key facilitator of oxidative stress.[39, 131]. While the current mechanism inducing neuroinflammation in MSA is unknown, it is hypothesized that potentially rare variants of inflammation-associated genes may increase susceptibility to such neuroinflammation.[39, 131] Notably, it has previously been shown that myeloperoxidase is involved in the neuroinflammation and neurotoxicity of MPTP induced Parkinson’s disease, indicating a potentially neuroprotective role of myeloperoxidase inhibition. In a transgenic mouse model, myeloperoxidase inhibition has several profound effects. Primarily, it has the ability to protect neurons susceptible to oxidative stress in the substantia nigra pars compacta, cerebellar cortex, striatum, pontine nuclei and inferior olives.[131] Secondly, resonating with the results of minocycline administration, myeloperoxidase inhibition reduces the amount of microglial activation, though notably does not affect astrogliosis.[131] Finally, it results in a decrease of alpha-synuclein aggregates located intracellularly, suggesting a potential therapeutic role of mitigating inflammation and oxidative stress. This reduction of alpha-synuclein aggregates occurs in a dose-dependent fashion, with higher doses of a myeloperoxidase inhibitor corresponding to larger declines in alpha-synuclein positive GCIs and increases in neuronal survival in the substantia nigra pars compacta and striatum.[131] Phenotypically, the reduction of motor dysfunction suggests a “partial reversal of oligodendroglial alpha-synuclein nitration and aggregation.” [131]

Role of the Ubiquitin-proteasome system (UPS)

In addition to the mechanisms of neuroinflammation and neurotoxicity, the role of protein turnover through the ubiquitin-proteasome system (UPS) and its association with MSA

pathophysiology has garnered growing interest within recent years. Previous investigations of alpha-synucleinopathies like PD have illuminated the role of UPS dysfunction.[132] Specifically occurring in the substantia nigra, the failure of the UPS correlates with the presence of Lewy bodies seen in PD.[132]

Alpha-synuclein is degraded by either one of two cellular mechanisms: autophagy or proteasomal machinery.[133] The former entails a lysosomal pathway forming autophagosomes, which utilize autophagosomal protein markers, *LC3* and a ubiquitin binding protein, *p62*, to allow entry of polyubiquitinated proteins, targeted for cellular destruction, inside the autophagosomes.[133] By investigating pathways used for oligodendroglial acquisition of alpha-synuclein accumulations in seven MSA cases, studies have detected *LC3*-positive vesicles manifesting an association with the alpha-synuclein aggregates located within GCIs. Given that *LC3* is an autophagy lysosomal pathway protein marker, this suggests a possible upregulation of this pathway in MSA pathophysiology.[133] Specifically, it was noted that only some of the GCIs were *LC3* positive, suggesting that increased activity of the autophagy pathway occurs after alpha-synuclein aggregations have already formed. [133] Further, there is evidence of “genuine cross-talk” between the autophagy and UPS pathways, which may suggest a simultaneous downregulation of the proteasomal pathway in MSA pathogenesis.[133–135] While a mechanism for such communication is under scrutiny, studies have demonstrated that a reduction in UPS pathway activity leads to increased stress of the endoplasmic reticulum due to an accumulation of aggregated ubiquitinated proteins. This unfolded protein response (UPR), creates a pathway between the endoplasmic reticulum and cell nucleus, whereby transcription is upregulated for genes that activate the autophagy lysosomal pathway. [134] Thus, while a number of neurodegenerative disorders have been associated with a decrease in UPS pathway activity, this may trigger a corresponding rise in the autophagy pathway. [133, 136] With a potentially interdependent system between autophagy and proteasomal pathways, it is thought that while compensatory changes can be made in attempt to maintain a necessary protein degradation balance, perturbations in either system can have pronounced adverse effects.[133, 136]

In addition to testing the role of UPS dysfunction and MSA pathogenesis *in vitro*, transgenic mouse models have been designed to further our understanding. One study validated that the UPS is the primary degradation pathway for alpha-synuclein under normal conditions *in vivo* by using transgenic mice expressing human alpha-synuclein.[135] However, when there was an abundance of alpha-synuclein within human alpha-synuclein transgenic mice due to a dysfunctional UPS, the autophagy lysosomal pathway was activated, presumably as a compensatory mechanism. [135] Further, there was a well-established pattern of this altered pathway regulation sequence occurring with a greater frequency in aged mice. This may suggest that increased age, in conjunction with an elevated alpha-synuclein burden, is a risk factor for increased proteasomal pathway dysfunction, a mechanism that fits in an age associated disorder.[135] With consistently increased alpha-synuclein levels, the UPS pathway may be disrupted, and despite compensatory efforts of the autophagy pathway to upregulate protein degradative functions, a vicious cycle ensues, culminating in accumulation of alpha-synuclein in GCIs and oligodendroglial cell death.[137]

Additional studies in transgenic mice have explored the phenotypic modifications that occur with UPS dysfunction. In mice expressing human oligodendroglial alpha-synuclein, the proteasomal pathway was inhibited via induction of systemic proteasome inhibition (PSI). [137] Notably, PSI activation resulted in motor dysfunction, which was directly associated with neurodegeneration in the striatonigral and olivopontocerebellar systems of these transgenic mice. In contrast, mice expressing human oligodendroglial alpha-synuclein but lacking PSI induction, exhibited no motor deficits and an absence of neuronal loss in corresponding regions.[137] Moreover, systemic application of PSI in transgenic mice resulted in selective neurodegeneration of striatonigral and olivopontocerebellar systems, while all surrounding regions were intact, resonating with human MSA's affected regions. [137]

In further elucidating the mechanism, it is evident that PSI treatment in the transgenic mice induced aggregation of human alpha-synuclein located within oligodendroglia, as manifested by GCIs. This may have led to myelin degeneration, axonal swelling, and mitochondrial enlargement, a key indicator of mitochondrial stress. Such modifications, identical to MSA neuropathological findings, suggest that UPS dysfunction plays a critical role in the mechanism of MSA pathogenesis.[137]

The dynamic behind key players: neurotoxicity, oxidative stress and the UPS

To connect several key findings regarding the molecular mechanisms of MSA pathogenesis, it is perhaps necessary to analyze the relationship between the UPS and autophagy pathways with oxidative stress. Recently, investigation of the ubiquitin homologue, *SUMO-1*, has been found within "discrete subdomains" of alpha-synuclein inclusion bodies of MSA brain tissue.[138] Interestingly, a co-localization was observed between a lysosomal subset and *SUMO-1* in the brain tissue of MSA and PSP cases. As neurodegenerative diseases both exhibiting cytoplasmic inclusion bodies of alpha-synuclein and tau, respectively, these data may suggest an association between protein aggregation and *SUMO-1* through the lysosomal autophagy pathway.[138] As previous studies have strongly suggested a downregulation of UPS and an upregulation of the autophagy lysosomal pathway in MSA pathogenesis, *SUMO-1* could play a critical role in this process.[138]

How to Move Forward

Understanding the disease process is a critical step in the development of etiologic therapies; however, as is illustrated above there is still so much that is unknown about the molecular underpinnings of MSA. We believe that a priority in understanding more about this disease lies in defining and identifying the genetic basis of this disease. Such an understanding would not only provide a window into the etiology but will likely be very important in the development of biomarkers and in the early identification of pre-symptomatic patients.

Etiologically, familial studies, SNP and gene association studies, have underscored the role of genetics in MSA.[4, 8] Given that MSA is poorly responsive to levodopa and current treatment is primarily oriented to symptomatic relief, the importance of understanding the genetics and etiology of MSA is paramount, as there is an urgent need to move toward etiologic based therapies.[28] With very limited reliable information regarding genetic

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mutations or alterations in gene dosage as a cause of MSA, the search for novel risk genes, which may be in the form of common variants or rare variants, is the logical nexus for MSA research.[1] Among previous investigations that have studied the role of potential environmental risk factors, some have reported that MSA patients have been exposed to environmental insults more than controls.[47] However, the feasibility of pursuing further study in this domain is challenging in that it remains difficult to identify and quantify the numerous possible toxicant exposures that may contribute to MSA pathogenesis.[47] Conversely, the pursuit of genetic risk and causative loci is scientifically tractable. With the availability of next generation methods, including genome wide association and second generation sequencing, the ability to acquire meaningful data and inform clinical diagnosis is clear from previous examples.[14]

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We believe that it is critical to undertake a comprehensive genetic analysis of MSA. In the first instance, the most tractable method, both in terms of cost and interpretation, is genome wide association using SNP array methods. This method has provided valuable insight into the genetic basis of hundreds of diseases, including the related disorders PD and PSP.[80, 139, 140] In all likelihood, the application of this method in a well-powered cohort of MSA patients would yield novel and informative associations. Given MSA's shared alpha-synuclein pathology and clinical features with PD, one can hypothesize substantial overlap with results from the PD GWAS. Understanding this overlap, and importantly identifying where there are differences, has the potential to shed key insight into the pathogenesis of both diseases, an understanding that is critical in the development of etiologic-based therapeutics.

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Notably, the application of GWA to a well powered cohort of MSA patients will have several powerful outputs outside of simply identifying risk variants. First, the use of approaches such as GCTA will allow an assessment of the heritable component of MSA. [122] This method allows the user to assess the heritable component of a trait using genotype data, effectively by examining the level of genetic sharing between cases compared to controls. [122] This method has been successfully applied in diseases such as PD, where the heritable component attributable to common variants was estimated to be 30%.[141]. This is a critical need, not only does the field need to know whether there is a substantial genetic component to this disease, but it would also establish how large that component is, and importantly when we have exhausted the pool of genetic risk. Notably this method can also be used to estimate shared genetic risk across diseases; an obvious first step would be to examine if MSA-C and MSA-P share a genetic component, although admittedly this may be difficult to achieve given the relatively small number of cases available. However, this method could also be used to examine whether there exists a shared genetic risk component for MSA and PD, or other neurodegenerative diseases.

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We believe that the application of whole exome sequencing in MSA is also a critical next step. As with any new technology, there are some drawbacks of exome sequencing that must be addressed: firstly, current capture efficiency is not complete, meaning that the remaining exomic regions are not captured nor sequenced. Secondly, given that exome sequencing only targets coding regions, intronic regions involved in gene regulation or expression are not detected. Further, exome sequencing cannot reveal all genomic structural variation, given its

bias to coding regions only.[142] Lastly, as a research tool in its infancy, unlikely to be clinically available for several years, the financial investment towards reagents and equipment cannot be overlooked, although costs have decreased exponentially within the last couple of years.

Despite these limitations, the exomic approach has not only helped identify and properly diagnose certain diseases, as in an atypical case of Wolfram syndrome, as well as Freeman-Sheldon syndrome [143–145], but holds promise for finding both rare and common variants among patients with known clinical conditions like MSA. While diseases with classic Mendelian forms of inheritance serve as ideal candidates for exome sequencing, it is important to realize that complex disorders, provided that sample sizes are sufficient, are amenable as well, such as in the identification of *TREM2* variants as risk factors for Alzheimer's disease.[146] While studying potentially causative genetic loci of complex diseases, it is also important to acknowledge the concept of variable expressivity, which is considered the “rule” rather than “exception”; hence, even among highly penetrant mutations, phenotypic variation may result in discordance among genotype-phenotype assessments.[14] Reviewing the results of GWAS data for common diseases, it is believed that most of the heritability behind complex traits is unlikely to be attributed to common variants with mild effects; rather, a “significant proportion” of the heritability associated with complex diseases is likely due to rare variants with large effect sizes.[147, 148] While this is with respect to common, complex diseases like Type II Diabetes, these same principles may apply to rare diseases as well; the key is then to find those variants, which are incredibly rare among such a unique disorder like MSA, and requires extensive samples sizes.

For any comprehensive genetic method aimed at understanding the basis of MSA, significant challenges exist outside of the application of the method. These challenges center on the collection of a sufficient number of MSA cases with consistent diagnostic criteria. As verification of definitive MSA can only be confirmed upon autopsy, obtaining adequate sample sizes is a major obstacle in the experimental process.[2] Moreover, with the extensive clinical and pathological variability among MSA cases, an even larger number sample size is required to adequately detect any true positive associations.

As the minor allele frequency (MAF) demonstrates an inverse linear relationship with a required sample size, in which $1/\text{MAF}$ is directly proportional to the sample size, it is clear that substantially large cohorts are the most promising towards finding such rare variants. [122] However, sample size exhibits a quadratic relationship, $1/(\text{OR}-1)$ with the odds ratio, which is necessary for association detections. Hence, previous association studies (measured by OR) have all required a significantly larger sample size than next-generation sequencing (detection measured by MAF) since sample size is much more strongly affected by OR than MAF. [122] Therefore, next generation sequencing serves as an incredibly valuable tool even when cohort numbers are in the hundreds (vs. thousands), and is thus more likely to be lucrative than an association study with the same number of samples.

Furthermore, another key consideration is that the phenotypic classification of MSA has two types of presentation. This suggests that the genetic analysis of MSA may require the

parsing of subtypes, again with adequate numbers of patients, to yield distinct phenotypic correlations. While only speculation at this point, it is possible we may find rare or common variants specific to each MSA subtype, which may explain phenotypic discrepancies through genotypic variation and explain population variability as well. [46]

Regardless of the approach a critical need for success in any modern genetic investigation of MSA will require extensive scientific collaboration. The disease is rare enough that no single group can collect sufficient cases on its own. The field will not progress without pooling of clinical resources. The creation of an international collaborative framework, which can efficiently and easily share resources, the burden of analysis, and rapidly disseminate results should be the primary goal of any entity wishing to further research into the genetic basis of MSA. While these are considerable challenges, we would predict that progress in this disease will be much delayed until we move forward in our genetic understanding of this disease.

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Panel 1: key technological and analytical tools used in human genetics

GENOME WIDE ASSOCIATION (GWA)

Definition

A GWA study uses extremely dense genome wide genotyping to identify associations between genetic loci and the presence or absence of a trait. The goal is to identify genomic regions that contain risk alleles and this is done in a largely unbiased manner (i.e. without consideration of gene function or position). This is typically accomplished using millions of common genetic variants genotyped in large series of cases and controls.

Application

Used primarily in the identification of risk loci for diseases and traits, it is largely limited to the identification of common risk alleles and explicitly tests the common disease common variant hypothesis.

Limitations

In general GWA requires many thousands of cases and controls to reliably detect effects. Independent replication of identified loci is required. Does not reliably detect rare risk alleles. Identifies genetic regions that contain risk alleles, but does not identify either the causal risk allele or the affected gene.

Cost and Use

Current cost ~\$200 per sample. Since initial use in 2005 GWA has been widely adopted and is still used extensively today.

LINKAGE AND POSITIONAL CLONING

Definition

Linkage and positional cloning served as the critical methods in the identification of mutations that caused single gene (monogenic) disorders. Traditionally linkage analysis was performed in families with an obviously inherited disease. Polymorphic (variable) markers were run throughout the genomes of member of the family in order to identify regions of the genome that segregated with disease. The inference from this result was that a disease-segregating region was likely to contain the disease causing mutation. Linkage was performed using 200-800 polymorphic markers spaced throughout the genome, although more recently this has been replaced by the use of SNP panels of hundreds of thousands of variants. Following the identification of positive linkage gene candidates within that region were sequenced to identify the causal mutation (this portion of the experiment was termed positional cloning).

Application

Used in the identification of disease causing mutations in highly informative (but usually rare) families

Limitations

These methods were quite slow, with successful linkage and positional cloning projects often taking years. In general families that were informative enough for this method are extremely rare, and in particular for a late onset disease, challenging to collect (because multiple generations are required).

Cost and Use

Relatively inexpensive, however, these methods have been largely supplanted by the use of exome sequencing, which combines elements of linkage and sequencing

SECOND GENERATION SEQUENCING**Definition**

Second generation sequencing (SGS) represents a major advance in molecular genetics. This method allows the generation of extremely large amounts of DNA sequence data, including the routine sequencing of human genomes. Most commonly thus far in human genetics, this method has been used in the context of exome sequencing. This involves sequencing of the protein coding regions of the human genome.

Application

The principal application has been in the identification of disease causing mutations; exome sequencing allows an investigator to identify rare disease segregating mutations rapidly and quite efficiently. More recently there has been interest in applying this method to large groups rather than families in an attempt to identify risk alleles.

Limitations

The current methods are not able to easily detect certain types of variability (such as repeat expansions), and sequencing of certain parts of the genome (such as copy number variants) is unreliable.

Cost and Use

Within a research setting exome sequencing costs approximately \$500 per sample and whole genome sequencing \$1500 per sample; however, the price continues to decrease. Exome sequencing is widely used in genetics laboratories, but will likely be replaced by whole genome sequencing in short order.

Use of exome/genome sequencing in a clinical setting

This is becoming a more cost effective approach toward complex neurological diseases. However there are mixed opinions on reporting of mutations in genes that were not intended to be the target. For example, finding a *BRCA* mutation in a patient being investigated because of a neurological disease. Guidelines have been developed[149, 150] to aid clinicians and laboratories but this is a complicated matter and there is a large debate on clinical proceedings, ethical issues and consent.