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# *MAPT* haplotype diversity in multiple system atrophy

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## Abstract

**Introduction—**Multiple system atrophy (MSA) is a rare progressive neurodegenerative disorder. MSA was originally considered exclusively sporadic but reports of association with genes such as *SNCA*, *COQ2* and *LRRK2* have demonstrated that there is a genetic contribution to the disease. *MAPT* has been associated with several neurodegenerative diseases and we previously reported a protective association of the *MAPT* H2 haplotype with MSA in 61 pathologically confirmed cases.

**Methods—**In the present study, we assessed the full *MAPT* haplotype diversity in MSA patients using six *MAPT* tagging SNPs. We genotyped a total of 127 pathologically confirmed MSA cases, 86 patients with clinically diagnosed MSA and 1312 controls.

**Results—**We identified four significant association signals in our pathologically confirmed cases, two from the protective haplotypes H2 (MSA:16.2%, Controls:22.7%, p=0.024) and H1E (MSA:3.0%, Controls:9.0%, p=0.014), and two from the rare risk haplotypes H1x (MSA:3.7%, Controls:1.3%, p=0.030) and H1J (MSA:3.0%, Controls:0.9%, p=0.021). We evaluated the association of MSA subtypes with the common protective H2 haplotype and found a significant

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Authors' contribution

Study concept and design: Labbé, Heckman, Ross

Acquisition, analysis, or interpretation of data: All authors.

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difference with controls for MSA patients with some degree of MSA-C (MSA-C or MSA-mixed), for whom H2 occurred in only 8.6% of patients in our pathologically confirmed series (P<0.0001).

**Conclusions—**Our findings provide further evidence that *MAPT* variation is associated with risk of MSA. Interestingly, our results suggest a greater effect size in the MSA-C compared to MSA-P for H2. Additional genetic studies in larger pathologically confirmed MSA series and meta-analytic studies will be needed to fully assess the role of *MAPT* and other genes in MSA.

### Keywords

Multiple system atrophy; MAPT; association study; genetics

## 1. Introduction

Multiple system atrophy (MSA) is an adult-onset neurodegenerative disorder characterized by variable degrees of autonomic dysfunction, parkinsonism and cerebellar ataxia. The pathological hallmarks of MSA are α-synuclein-positive glial cytoplasmic inclusions (GCIs) which are required for definitive diagnosis [1]. The disease is considered sporadic and rare with prevalence rates ranging from 1.9 to 4.9 per 100,000 people [2]. Treatment options are limited and strictly supportive, and patients with MSA have a relatively poor prognosis compared to patients with Parkinson's disease (PD) with median survival around 9 years from initial symptoms [3, 4]. There is no cure for MSA and poor understanding of the disease etiology is one of the greatest contributors to lack of such treatment.

Recently, Tsuji *et al.* identified variants in the *COQ2* gene as risk factor for MSA in familial and population based Japanese series [5]. Established PD genes such as *SNCA* [6] and *LRRK2* [7] have also been implicated in the risk to MSA. Recently, the *GBA* gene has also been associated to MSA [8]. The microtubule associated protein tau gene (*MAPT*) has been identified as a risk factor for many neurodegenerative diseases. The *MAPT* gene sits in a locus of extended linkage disequilibrium characterized by two main haplotypes: H1 and H2. The common H1 risk haplotype has been associated to increased risk of several neurodegenerative diseases such as PD [9], progressive supranuclear palsy (PSP) [10], and corticobasal degeneration (CBD) [11] in genome-wide association studies (GWAS). The H1 and the protective H2 haplotypes have traditionally been tagged by a single variant but Pittman *et al.* [12] and others [13] demonstrated that the diversity at the locus far exceeds the simplistic H1/H2 dichotomy. Pittman and colleagues used six *MAPT* tag SNPs to capture more than 95% of the haplotype diversity at the locus and define over 20 H1 subhaplotypes. H1 subhaplotypes have been independently implicated in increased risk of neurodegenerative diseases, for example, H1 haplotype C (H1C) is associated with PSP [12] and AD [14, 15].

We previously detected an association of the *MAPT* H1 haplotype in a small study of 61 pathologically confirmed MSA cases and 409 healthy controls (p=0.016).[16] Having more than doubled our sample size, we decided to explore the full haplotype diversity in our MSA series. We genotyped six *MAPT* haplotype tagging SNPs in 213 cases, including 127 pathologically confirmed cases, and 1312 controls. We detected a protective effect of the H2 haplotype in our pathological series, and this was particularly evident when considering the

MSA-mixed subtype only or the MSA-mixed and MSA-C subtypes. A novel protective association with the H1E subhaplotype and novel risk associations with the rare H1x and H1J subhaplotypes were also detected in pathologically confirmed cases.

## 2. Methods

### 2.1. Study subjects

A total of 213 MSA patients (127 pathologically confirmed and 86 clinically diagnosed) and 1312 controls were included in this study. Of these, 44 pathologically confirmed MSA were part of our previous study [15]. The pathologically confirmed MSA patients were considered to be our primary series due to the definitive diagnosis, with the clinical MSA patients serving as a secondary exploratory series. The pathologically confirmed MSA patients were all cases received at the Mayo Clinic Jacksonville brain bank for neurodegenerative disorders and examined at Mayo Clinic Jacksonville by our neuropathologist (DWD) between 1998 and 2015. These cases were designated as MSA parkinsonian type (MSA-P) (n=51), MSA cerebellar type (MSA-C) (n=20), and MSA-mixed (n=56) based on pathology. MSA-C cases have predominant olivopontocerebellar degeneration, MSA-P cases have predominant striatonigral degeneration and MSA-mixed cases have equal pathology in olivopontocerebellar and striatonigral systems. Degeneration is defined by neuronal loss and gliosis and all cases have GCI and variable neuronal cytoplasmic inclusions (NCI) in both systems. Clinically diagnosed MSA patients were diagnosed at the Mayo Clinic in Jacksonville, FL (N=50) and Rochester, MN (N=36) where diagnosis of MSA was made using current consensus criteria [1]. Of the 86 clinically diagnosed MSA patients, 78 were probable MSA and 8 were possible MSA. Among the clinical cases, 52 are MSA-P, 24 MSA-C and 10 have a mixed phenotype of MSA with parkinsonism and cerebellar ataxia. All control individuals were free of personal or familial history suggestive of parkinsonism, cerebellar ataxia or autonomic failure and were seen at the Mayo Clinic in Jacksonville, FL (N=881) or Rochester, MN (N=431). All individuals were unrelated within and between sample groups. All subjects are unrelated non-Hispanic Caucasians of European descent. Characteristics of patients with pathologically confirmed MSA, clinically diagnosed MSA, and controls are summarized in Table 1. The Mayo Clinic Institutional Review Board approved the study and all subjects or legal next of kin provided written informed consent.

### 2.2. Genetic analysis

Genomic DNA was extracted from peripheral blood monocytes or brain tissue using the standard protocols [17]. Six tagging SNPs were chosen to assess the most common *MAPT* subhaplotypes as described previously [12, 18]. The genotyping of *MAPT* haplotype tagging variants rs1467967, rs242557, rs3785883, rs2471738, rs8070723 (the H2-tagging variant), and rs7521 was performed using TaqMan SNP genotyping assays on an ABI 7900HT Fast Real-Time PCR system (Applied Bio-systems, Foster City, CA, USA) according to the manufacturer's instructions (primer sequences are available upon request). Genotype calls were made using Taqman Genotyper Software v1.3 (Applied Bio-systems, Foster City, CA, USA). The genotype call-rate was 100%. There was no evidence of a departure from Hardy-Weinberg equilibrium in study controls for any of the six *MAPT* variants (all $P \geq 0.01$ after Bonferroni correction).

### 2.3. Statistical analysis

All analysis was performed separately for the primary group of pathologically confirmed MSA patients, the clinically diagnosed MSA patients, and the combined group of pathologically confirmed and clinically diagnosed patients. The association between each individual *MAPT* variant and risk of MSA was evaluated using a logistic regression model adjusted for age (age at death for pathologically confirmed MSA patients and age at blood sample for clinically diagnosed MSA patients and controls) and gender. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated, and each *MAPT* variant was examined under an additive model (i.e. effect of each additional minor allele). Associations between a six-variant *MAPT* haplotype and risk of MSA were examined using the R haplo.score and haplo.glm functions, where haplotypes occurring in less than 0.5% of subjects were excluded and adjustments were made for age and gender as previously described. Specifically, using haplo.score, we performed score tests of association that compared the frequency of each individual haplotype between MSA patients and controls, while using haplo.glm we utilized logistic regression models to obtain ORs and 95% CIs in comparison to a common reference haplotype. The common H1C haplotype was chosen as the reference category as it was the haplotype that occurred at a frequency of greater than 10% was not significantly associated with risk of MSA in any of the series. We did not make any adjustment for multiple testing in this exploratory analysis owing to the low power we had to detect associations with MSA; p-values ≤0.05 were considered as statistically significant. As a result of this lack of adjustment, it is important to highlight that our findings require validation. All statistical analysis was performed using R Statistical Software (version 3.0.2; R Foundation for Statistical Computing, Vienna, Austria).

## 3. Results

Single variant associations between individual *MAPT* variants and risk of MSA are displayed in Table 2. When considering only the pathologically confirmed MSA patients, significant associations with risk of MSA were observed for rs242557 (OR: 1.32, P=0.033), rs3785883 (OR: 1.44, P=0.020) and rs8070723 (OR: 0.69, P=0.029). When comparing all MSA patients to controls, there was a significant association between rs242557 and risk of MSA (OR: 1.25, P=0.031). None of the *MAPT* variants were associated with risk of clinically diagnosed MSA (all P≥0.052, Table 2).

We evaluated the association between *MAPT* haplotype and MSA risk and detected 24 haplotypes that occurred with a frequency >0.5% (Table 3). When comparing the primary series of pathologically confirmed MSA patients to controls, significant protective associations were observed for the H2 (MSA: 16.2% Controls: 22.7%, P=0.024) and H1E (MSA: 3.0%, Controls: 9.0%, P=0.014) haplotypes, while significant risk associations were observed for the rare H1J (MSA: 3.0%, Controls, 0.9%, P=0.021) and H1x (MSA: 3.7%, Controls: 1.3%, P= 0.030) haplotypes. For the secondary series of clinically diagnosed MSA patients, a significant difference compared to controls was observed for the H1U haplotype (MSA: 5.5%, Controls: 2.4%, P=0.013). When combining the pathological and clinical MSA series, significant associations with risk of MSA were observed for H1U (MSA: 4.2%, Controls: 2.4%, P=0.049) and H1x (MSA: 3.2%, Controls: 1.3%, P=0.049).

An analysis of the association between *MAPT* haplotype and MSA subtypes was not feasible owing to the small sample sizes of the individual subtypes. However, in an analysis that should be considered as exploratory owing to the small sample sizes of some of the subtypes, we did examine the association between the H2 haplotype (as defined by the rs8070723 variant) and risk of MSA subtypes, and these results are shown in Table 4. When evaluating just the pathologically confirmed MSA patients, significant protective associations for H2 were observed for the MSA-C subtype (OR: 0.27, P=0.030), the MSA-mixed subtype (OR: 0.33, P=0.001) and the combined group of MSA-C and MSA-Mixed patients (OR: 0.32, P<0.0001). No associations with MSA subtypes were observed when considering the clinically diagnosed MSA patients alone (Table 4). When combining the pathologically confirmed and clinically diagnosed MSA patients, there was a significant protective effect for the H2 haplotype when considering MSA-mixed patients (OR: 0.50, P=0.008) and the combined group of MSA-C and MSA-Mixed patients (OR: 0.60, P=0.007).

## 4. Discussion

The *MAPT* gene has been implicated in several neurodegenerative diseases including synucleinopathies such as PD [9] and DLB [19]. We previously reported a protective association of the haplotype H2 in our screening of 61 patients with pathologically confirmed MSA and 409 controls [16]. Here, we expand on this finding by studying 213 MSA patients including 127 pathologically confirmed cases and 1312 controls collected at Mayo Clinic. While these numbers may not initially appear impressive in the context of population-based association studies for more common diseases, they represent one of the largest pathologically confirmed series of MSA patients in the world.

Our results provide further evidence that *MAPT* variation is associated with risk of MSA. Specifically, in our primary pathologically confirmed MSA series, three of the six individual *MAPT* haplotype tagging SNPs were significantly associated with MSA risk. Additionally, in haplotype analysis, we confirmed the association with MSA for the protective haplotype H2, and identified novel associations to haplotypes H1E, H1x and H1J. Although none of these four significant haplotypes in the pathologically confirmed MSA series would have survived a Bonferroni correction for multiple testing, it is worth noting that when utilizing the less conservative false-discovery rate approach, we would expect three of these four significant (P≤0.05) associations to be real. Surprisingly, we detect a protective association to subhaplotype H1E which illustrates that disease risk is more complicated than the H1 risk vs H2 protective haplotypes and that there is a need for complete haplotyping to fully understand variability at the *MAPT* locus.

Additionally, several significant associations were observed when examining the association between the H2 haplotype (as measured by the rs8070723 variant) and MSA subtypes. Specifically, subtypes with prominent cerebellar dysfunction (i.e. MSA-C or MSA-mixed) had a lower frequency of the H2 haplotype, and for the pathologically confirmed series these findings were highly significant with frequencies under 9% and would remain significant after correction for multiple testing. Considering that PD is highly associated with *MAPT*, one would expect MSA-P to be associated. Our data suggests that MSA-C and MSA-P could

have different genetic risk at least concerning *MAPT*. The low prevalence of MSA has mostly precluded genetic studies from looking at MSA subtypes but interestingly, GBA variants have been found to be associated with MSA-C in a recent multicentric study [8]. Protein tau can be found in GCIs in patients with MSA [20]. It is also found at high levels in the cerebro-spinal fluid of MSA patients compared to PD patients [21] and there is at least one case report of a patient with MSA-C and tau pathology [22]. However, the role of tau in MSA and its different subtypes needs to be further explored.

MSA is a rare disease with the genetic risk most likely coming from rare variants to some degree. Federoff *et al.* used common SNPs and imputed data to calculate the heritability of MSA namely phenotypic variation attributable to genetic variation [23]. In their pathologically confirmed cases (N=291), the heritability was almost 0 when using genotyped data and when using imputed data it was 5.8% suggesting rare variants have a major contribution to the heritability. This is in line with our finding of associations with rare haplotype H1x and H1J.

It is worth commenting on some of the discordant findings between the pathological and clinical MSA series. For instance, of the four haplotypes that were significantly associated with disease in the primary pathologically confirmed MSA series, only one of these (H1x) was observed at a similar frequency in the clinical MSA series. The results of MSA subtype analysis involving the H2 haplotype also differed between the two series, with a strong protective association for MSA-C observed only in the pathological series. One simple explanation for this between-series heterogeneity is that relatively speaking, the sample sizes of both series are relatively small, and this naturally results in a high degree of variability of haplotype frequencies and association estimates due to their lack of precision.

There are several limitations of this study. First, the number of MSA patients included is small for a genetic association study, and therefore the possibility of a false-negative finding is important to consider. Second and related to this, owing to the inherently low power of MSA genetic studies, we did not make any adjustment for multiple testing despite the relatively large number of statistical tests that were performed. Therefore, it is very important to highlight that our findings require validation. Indeed, 95% confidence limits for some of our odds ratio estimates (particularly those involving rare haplotypes) are relatively wide, which further underscores the need for replication and meta-analytic studies. Additionally, the clinical misdiagnosis rate of MSA can be high, with a notable proportion of patients who receive an initial diagnosis of MSA go on to develop DLB, PD or PSP [24]. This creates a degree of uncertainty when examining the association between *MAPT* haplotypes and clinically diagnosed MSA, and therefore caution is warranted when interpreting findings involving our clinically diagnosed MSA series. Finally, we cannot rule out the possibility that population stratification could have had an effect on our results. Larger studies of pathologically confirmed MSA patients will help resolve most of these issues and better define the role of *MAPT* haplotypic variation in susceptibility to MSA.

Undoubtedly, the next challenge in MSA genetics is to increase sample size, preferably of pathologically confirmed cases, to be able to identify variants with small effect size. It is likely that the sample sizes will never reach the numbers seen in studies that are occurring in

other more common diseases, but nevertheless, meta-analytic GWAS combined to whole exome/genome sequencing studies will be important tools to identify common and rare variants in MSA and its subtypes. Clarifying the role of *MAPT* and other genes in the etiology of MSA will improve clinical diagnosis and lead to better treatment.

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## Highlights

- In this association study, we identified four *MAPT* haplotypes associated to MSA.
- Haplotype H2 and H1E are protective and H1x and H1J are risk haplotypes.
- The *MAPT* gene is involved in the susceptibility to MSA.
- Follow-up sequencing studies should be attempted to pinpoint causal variants.

## Table 1

Subjects characteristics

| Variable | Pathologically confirmed MSA patients (N=127) | Clinically diagnosed MSA patients (N=86) | Controls (N=1312) |
|---|---|---|---|
| Age (years)[1] | 67 (47, 91) | 67 (45, 88) | 69 (45, 92) |
| Gender | | | |
| Male | 75 (59.1%) | 49 (57.0%) | 611 (46.6%) |
| Female | 42 (40.9%) | 37 (43.0%) | 701 (53.4%) |
| MSA subtype | | | |
| MSA-P | 51 (40.2%) | 52 (60.5%) | N/A |
| MSA-C | 20 (15.7%) | 24 (27.9%) | N/A |
| MSA-mixed | 56 (44.1%) | 10 (11.6%) | N/A |

The sample median (range) is given for age.

[1]Age at death is given for pathologically confirmed MSA patients and age at blood collection is given for controls and clinically diagnosed MSA patients. MSA=multiple system atrophy.

**Table 2**

Single variant associations with MSA

| | | | Pathologically confirmed MSA patients vs. controls | | | Clinically diagnosed MSA patients vs. controls | | | All MSA patients vs. controls | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Variant** | **MA** | **MAF in controls (%) (N=1312)** | **MAF in patients (%) (N=127)** | **OR (95% CI)** | **P** | **MAF in patients (%) (N=86)** | **OR (95% CI)** | **P** | **MAF in patients (%) (N=213)** | **OR (95% CI)** | **P** |
| rs1467967 | G | 32.4 | 33.5 | 1.06 (0.81, 1.39) | 0.68 | 25.0 | 0.70 (0.49, 1.00) | 0.052 | 30.0 | 0.91 (0.72, 1.13) | 0.38 |
| rs242557 | A | 36.8 | 43.7 | 1.32 (1.02, 1.70) | 0.033 | 40.7 | 1.16 (0.86, 1.56) | 0.34 | 42.5 | 1.25 (1.02, 1.53) | 0.031 |
| rs3785883 | A | 18.0 | 24.0 | 1.44 (1.06, 1.97) | 0.020 | 14.0 | 0.74 (0.48, 1.16) | 0.19 | 20.0 | 1.14 (0.88, 1.48) | 0.32 |
| rs2471738 | T | 20.7 | 24.4 | 1.22 (0.91, 1.64) | 0.18 | 16.9 | 0.79 (0.53, 1.17) | 0.23 | 21.4 | 1.04 (0.81, 1.32) | 0.77 |
| rs8070723 | G | 23.0 | 16.9 | 0.69 (0.49, 0.96) | 0.029 | 26.7 | 1.21 (0.86, 1.71) | 0.28 | 20.9 | 0.89 (0.69, 1.14) | 0.34 |
| rs7521 | A | 46.6 | 45.7 | 0.97 (0.75, 1.25) | 0.81 | 45.9 | 1.05 (0.77, 1.42) | 0.77 | 46.5 | 1.00 (0.81, 1.22) | 0.98 |

ORs, 95% CIs, and p-values (P) result from logistic regression models adjusted for age and gender. ORs correspond to each additional minor allele. MSA=multiple system atrophy; MA=minor allele; MAF=minor allele frequency; OR=odds ratio; CI=confidence interval

**Table 3**

Association of *MAPT* haplotype with risk of MSA

| | | Pathologically confirmed MSA patients vs. controls | | | | Clinically diagnosed MSA patients vs. controls | | | All MSA patients vs. controls | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Haplotype** | **Sequence** | **Freq. in controls (%) (N=1312)** | **Freq. in patients (%) (N=127)** | **P** | **OR (95% CI) vs. H1C** | **Freq. in patients (%) (N=86)** | **P** | **OR (95% CI) vs. H1C** | **Freq. in patients (%) (N=213)** | **P** | **OR (95% CI) vs. H1C** |
| H1C | AAGTAG | 11.3 | 12.3 | 0.35 | 1.00 (reference) | 8.9 | 0.39 | 1.00 (reference) | 10.4 | 0.87 | 1.00 (reference) |
| H2 | AGGCGG | 22.7 | 16.2 | **0.024** | 0.65 (0.39, 1.08) | 26.1 | 0.32 | 1.39 (0.73, 2.64) | 20.2 | 0.27 | 0.91 (0.61, 1.36) |
| H1B | GGGCAA | 16.0 | 16.5 | 0.88 | 0.90 (0.54, 1.51) | 10.7 | 0.051 | 0.81 (0.38, 1.73) | 14.1 | 0.19 | 0.88 (0.57, 1.37) |
| H1E | AGGCAA | 9.0 | 3.0 | **0.014** | 0.34 (0.14, 0.84) | 12.6 | 0.11 | 1.71 (0.81, 3.62) | 7.2 | 0.47 | 0.86 (0.50, 1.46) |
| H1D | AAGCAA | 7.1 | 9.3 | 0.61 | 1.17 (0.60, 2.27) | 8.9 | 0.19 | 1.45 (0.65, 3.24) | 8.7 | 0.25 | 1.24 (0.73, 2.10) |
| H1I | GAGCAA | 4.4 | 4.3 | 0.85 | 0.77 (0.31, 1.92) | 5.6 | 0.54 | 1.52 (0.54, 4.26) | 5.0 | 0.61 | 1.04 (0.52, 2.10) |
| H1H | AGACAA | 4.1 | 5.5 | 0.37 | 1.14 (0.52, 2.52) | 4.1 | 0.81 | 1.18 (0.40, 3.45) | 4.8 | 0.63 | 1.18 (0.61, 2.28) |
| H1L | AGACAG | 3.0 | 5.0 | 0.079 | 1.51 (0.72, 3.18) | 0.9 | 0.12 | 0.30 (0.04, 2.32) | 3.3 | 0.73 | 1.10 (0.54, 2.24) |
| H1M | GAGCAG | 2.9 | 2.0 | 0.89 | 0.83 (0.30, 2.28) | 1.1 | 0.29 | 0.53 (0.10, 2.91) | 1.5 | 0.46 | 0.73 (0.30, 1.79) |
| H1U | AAGCAG | 2.4 | 2.6 | 0.48 | 1.04 (0.36, 3.03) | 5.5 | **0.013** | 2.80 (1.01, 7.79) | 4.2 | **0.049** | 1.67 (0.82, 3.38) |
| H1O | AAACAA | 2.3 | 3.3 | 0.29 | 1.14 (0.40, 3.21) | 4.0 | 0.44 | 1.69 (0.53, 5.33) | 3.7 | 0.20 | 1.36 (0.61, 3.02) |
| H1y | AAATAG | 1.6 | 2.1 | 0.21 | 1.21 (0.35, 4.19) | 0 | 0.37 | N/A[1] | 1.7 | 0.67 | 0.94 (0.29, 3.11) |
| H1P | GGGTAG | 1.5 | 0.5 | 0.30 | 0.32 (0.04, 2.56) | 0 | 0.18 | N/A[1] | 0.3 | 0.10 | 0.23 (0.03, 1.80) |
| H1x | GAATAG | 1.3 | 3.7 | **0.030** | 2.10 (0.76, 5.82) | 2.6 | 0.48 | 2.31 (0.60, 8.89) | 3.2 | **0.049** | 2.25 (0.95, 5.29) |
| H1F | GGACAA | 1.2 | 1.8 | 0.27 | 1.60 (0.46, 5.56) | 0 | 0.2 | N/A[1] | 1.1 | 0.95 | 0.98 (0.27, 3.52) |
| H1v | GGATAG | 1.2 | 1.7 | 0.65 | 0.50 (0.08, 3.28) | 0 | 0.18 | N/A[1] | 0.4 | 0.23 | 0.34 (0.05, 2.39) |
| H1R | AGGTAG | 1.1 | 1.6 | 0.90 | 1.10 (0.20, 6.00) | 1.4 | 0.91 | 1.64 (0.32, 8.29) | 1.7 | 0.83 | 1.37 (0.40, 4.66) |
| H1G | GAACAA | 1.1 | 0 | 0.80 | N/A[1] | 0 | 0.95 | N/A[1] | 0 | 0.84 | N/A[1] |
| H1Q | AAGTAA | 1.0 | 1.9 | 0.35 | 1.52 (0.47, 4.97) | 0.6 | 0.58 | 0.78 (0.09, 6.52) | 1.3 | 0.72 | 1.26 (0.44, 3.61) |
| H1J | AGGCAG | 0.9 | 3.0 | **0.021** | 3.88 (1.26, 11.91) | 0 | 0.64 | N/A[1] | 1.7 | 0.17 | 2.29 (0.74, 7.08) |
| H1S | GGGCAG | 0.9 | 0.5 | 0.84 | N/A[1] | 1.3 | 0.93 | 2.04 (0.40, 10.40) | 0.8 | 0.93 | 0.82 (0.14, 4.84) |
| H1z | GAGTAG | 0.8 | 1.6 | 0.55 | 1.33 (0.27, 6.53) | 2.1 | 0.45 | 4.03 (0.76, 21.39) | 1.9 | 0.37 | 1.94 (0.55, 6.85) |

| | | Pathologically confirmed MSA patients vs. controls | | | | Clinically diagnosed MSA patients vs. controls | | | All MSA patients vs. controls | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Haplotype** | **Sequence** | **Freq. in controls (%) (N=1312)** | **Freq. in patients (%) (N=127)** | **P** | **OR (95% CI) vs. H1C** | **Freq. in patients (%) (N=86)** | **P** | **OR (95% CI) vs. H1C** | **Freq. in patients (%) (N=213)** | **P** | **OR (95% CI) vs. H1C** |
| H1N | GGACAG | 0.6 | 1.2 | 0.14 | 2.34 (0.51, 10.77) | 0.3 | 0.49 | N/A[1] | 0.7 | 0.74 | 1.53 (0.28, 8.48) |
| H1K | AAACAG | 0.5 | 0.7 | 0.53 | 0.89 (0.10, 7.95) | 1.4 | 0.22 | 3.73 (0.68, 20.58) | 0.8 | 0.27 | 1.29 (0.26, 6.50) |

[1]Denotes a haplotype that occurred too rarely for odds ratio estimation. Haplotype-specific p-values result from score tests of association that were adjusted for age and gender, and where the association between the given haplotype and disease is being tested. ORs and 95% CIs given in comparison to the reference category of the common H1C haplotype result from logistic regression models with the previously described adjustments for age and gender. Note that the p-value (P) (which results from a test of association between the given haplotype and risk of MSA in comparison to all other haplotypes) and the OR and 95% CI (which result from a test of association between the given haplotype and risk of MSA in comparison to the common H1C haplotype) do not directly correspond to one another. Freq.= Frequency; MSA=multiple system atrophy; OR=odds ratio; CI=confidence interval.

## Table 4

Associations between the H2 haplotype (rs8070723) and MSA subtypes

| Series/MSA subtype | N | MAF (%) | OR (95% CI) | P-value |
|---|---|---|---|---|
| Controls | 1312 | 23.0 | 1.00 (reference) | N/A |
| Pathologically confirmed MSA-P patients | 51 | 29.4 | 1.38 (0.89, 2.13) | 0.15 |
| Pathologically confirmed MSA-C patients | 20 | 7.5 | 0.27 (0.08, 0.88) | 0.030 |
| Pathologically confirmed MSA-mixed patients | 56 | 8.9 | 0.33 (0.17, 0.64) | 0.001 |
| Pathologically confirmed MSA-P or MSA-Mixed patients | 107 | 18.7 | 0.77 (0.54, 1.10) | 0.15 |
| Pathologically confirmed MSA-C or MSA-Mixed patients | 76 | 8.6 | 0.32 (0.18, 0.56) | <0.0001 |
| Clinically diagnosed MSA-P patients | 52 | 25.0 | 1.11 (0.71, 1.73) | 0.66 |
| Clinically diagnosed MSA-C patients | 24 | 27.1 | 1.22 (0.65, 2.28) | 0.53 |
| Clinically diagnosed MSA-Mixed patients | 10 | 35.0 | N/A[1] | N/A |
| Clinically diagnosed MSA-P or MSA-Mixed patients | 62 | 26.6 | 1.20 (0.80, 1.80) | 0.38 |
| Clinically diagnosed MSA-C or MSA-Mixed patients | 34 | 29.4 | 1.37 (0.81, 2.30) | 0.24 |
| All MSA-P patients | 103 | 27.2 | 1.23 (0.90, 1.70) | 0.19 |
| All MSA-C patients | 44 | 18.2 | 0.74 (0.43, 1.27) | 0.28 |
| All MSA-Mixed patients | 66 | 12.9 | 0.50 (0.30, 0.84) | 0.008 |
| All MSA-P or MSA-Mixed patients | 169 | 21.6 | 0.92 (0.70, 1.21) | 0.55 |
| All MSA-C or MSA-Mixed patients | 110 | 15.0 | 0.60 (0.41, 0.87) | 0.007 |

ORs, 95% CIs, and p-values result from logistic regression models adjusted for age (age at death in pathologically confirmed MSA patients and age at blood collection in clinically diagnosed MSA patients and controls) and gender. ORs correspond to each additional H2 allele.

MSA=multiple system atrophy; MAF=minor allele frequency; OR=odds ratio; CI=confidence interval

[1]Logistic regression analysis was not performed owing to the small number of clinically diagnosed MSA-mixed patients.