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MAPT haplotype diversity in multiple system atrophy

Catherine Labbé [a], Michael G. Heckman [b], Oswaldo Lorenzo-Betancor [a], Melissa E. Murray [a], Kotaro Ogaki [a], Alexandra I. Soto-Ortolaza [a], Ronald L. Walton [a], Shinsuke Fujioka [a], Shunsuke Koga [a], Ryan J. Uitti [c], Jay A. van Gerpen [c], Ronald C. Petersen [d], Neill R. Graff-Radford [c], Steven G. Younkin [a], Bradley F. Boeve [d], William P. Cheshire Jr. [c], Phillip A. Low [d], Paola Sandroni [d], Elizabeth A. Coon [d], Wolfgang Singer [d], Zbigniew K. Wszolek [c], Dennis W. Dickson [a], Owen A. Ross [a, e, *]

[a] Department of Neuroscience, Mayo Clinic, Jacksonville, FL, 32224, USA
[b] Division of Biomedical Statistics and Informatics, Mayo Clinic, Jacksonville, FL, 32224, USA
[c] Department of Neurology, Mayo Clinic, Jacksonville, FL, 32224, USA
[d] Department of Neurology, Mayo Clinic, Rochester, MN, 55905, USA
[e] Mayo Graduate School, Mayo Clinic, Jacksonville, FL, 32224, USA

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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 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.

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.

* Corresponding author. Department of Neuroscience, Mayo Clinic Jacksonville, 4500 San Pablo Road, Jacksonville, FL, 32224, USA.
E-mail address: ross.owen@mayo.edu (O.A. Ross).

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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