

Mutation Analysis of *COQ2* in Chinese Patients with Cerebellar Subtype of Multiple System Atrophy

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Keywords

Chinese; *COQ2* variants; Multiple system atrophy; Mutation analysis.

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SUMMARY

Aims: Recently, mutations in *COQ2* encoding para-hydroxybenzoate-polyprenyl transferase have been identified to increase the risk of multiple system atrophy (MSA) in multiplex families and sporadic cases. The prevalence of *COQ2* mutations was showed to be higher in cerebellar subtype (MSA-C) than parkinsonism subtype (MSA-P). The aim of this study was to investigate the association between *COQ2* mutations and MSA-C in Chinese patients.

Methods: A Chinese cohort of 116 patients with MSA-C and 192 healthy control individuals were recruited. Sanger sequencing of *COQ2* was performed in all these subjects. **Results:** Two missense mutations (p.L402F and p.R173H) and one synonymous mutation (p.A32A) were detected in 3 patients, respectively. They were not found in the 192 controls as well as the 1000 Genomes Database. The p.L402F and p.A32A were novel. **Conclusion:** Our results indicated that *COQ2* tended to play a population-specific and subtype-depended role in conferring susceptibility to MSA.

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The first two authors contributed equally to this work.

Introduction

Multiple system atrophy (MSA) is a progressive neurodegenerative disorder characterized by various combinations of autonomic insufficiency, parkinsonism, cerebellar ataxia, and pyramidal dysfunction. The prevalence of MSA is estimated at 3.4 to 4.9 cases per 100,000 individuals, similar to that of motor neuron disease (MND) and Huntington disease (HD) [1]. Affecting both genders, the disease usually begins in middle age and progresses slowly, with a median survival of 9.3 years from the onset [2]. Clinically, MSA is classified into two subtypes: parkinsonism type (MSA-P), predominantly featured by parkinsonism, and cerebellar type (MSA-C), predominantly featured by cerebellar ataxia [2]. The MSA-P is more prevalent among the Caucasian patients [3,4], while MSA-C is more prevalent in Asian patients [5]. The treatment of MSA is intractable, and there is no adequate approach to halt the progression of the disease so far.

The neuropathologic change of MSA is characterized by cell loss, gliosis, and glial cytoplasmic inclusions (GCI) in many regions of central nervous system (CNS) [6]. However, the potential mechanism underlying MSA remains to be elucidated. During the past decades, MSA was widely conceived as a nongenetic disorder, because the vast majority of MSA cases reported were sporadic [7]. Nevertheless, several multiplex families with MSA were described over the last few years [8,9]. A breakthrough in our understanding of MSA was the findings that variants within *SNCA* conferred susceptibility to MSA [10,11]. Recently, a multiple-center study identified that *COQ2* variants were associated with the increased risk of MSA in patients of Japan, Europe, and North America [12]. The *COQ2* gene was subsequently screened in MSA patients from South Korea, UK, Germany, and southwest China [13–17]. However, these studies presented conflicting results about the association of *COQ2* variants with MSA in different populations and different MSA subtypes. In this study, we recruited

116 MSA-C cases and investigated the association between *COQ2* variants and these cases.

Materials and Methods

Subjects

A cohort of 116 unrelated sporadic MSA-C patients was recruited between June 2008 and December 2013. The cohort consisted of 62 males and 54 females. No consanguineous marriage was found in any family. The mean age of onset was 51.27 ± 7.72 years, and the average age at examination was 53.17 ± 7.81 years. All the patients were neurologically evaluated by at least two senior neurologists. Brain magnetic resonance imaging (MRI) was performed in all the cases. The diagnosis of MSA was made according to the current consensus criteria by Gilman *et al.* [2]. The predominant symptom of all cases in our cohort was cerebellar ataxia including gait ataxia and cerebellar dysarthria. Among them, 79 patients (79/116, 68.1%) were diagnosed as probable MSA-C, and 37 patients (37/116, 31.9%) were diagnosed as possible MSA-C. Brain MRI showed severe cerebellar atrophy in all cases and pontine atrophy in 57 cases (57/116, 49.1%). The details of the onset symptoms and follow-up symptoms were listed in Table S1. All cases were of Han Chinese descent and the majority of them come from southeast of China (Zhejiang, Jiangsu, Shanghai, Jiangxi, and Anhui Province). In addition, 192 healthy individuals matched by gender, age, and ethnicity were enlisted as normal controls. This study was approved by the local ethics committees. Written informed consent was obtained from each participant.

Screening of *COQ2* Mutations

Genomic DNA was extracted from peripheral blood using a QIA-amp DNA blood minikit (QIAGEN, Hilden, Germany). For mutation analysis of *COQ2*, fragments encompassing the coding regions and flanking introns were amplified by polymerase chain reaction (PCR). The primers used in this study and the annealing temperatures were listed in the Table S2. The amplified products were purified, followed by Sanger sequencing with ABI 3730 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). The resulting sequences were compared against the NCBI human reference DNA sequence of *COQ2* (NM_015697).

Statistical Analysis

The genotypes in MSA patients and controls were compared using chi-square test or Fisher's exact test. All statistical analyses were carried out by SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Difference was considered significant when the *P* value was less than 0.05 ($P < 0.05$).

Results

Genetic Investigations

After the Sanger sequencing of *COQ2* in 116 unrelated MSA-C patients, totally 8 variants were identified. Among these variants, c. 196G>T (p.L66V), c. 894T>C (p.D298D), and c. 990C>T

(p.S330S) were common and present in 1000 Genomes Database. Furthermore, we found 2 missense mutations including c. 1206G>C (p.L402F) (Figure 1A) and c. 518G>A (p.R173H) (Figure 1B), and one synonymous mutation c. 96G>A (p.A32A) (Figure 1C) in one single patient each. The p.R173H was recently reported in a southwest Chinese study, while the p.L402F and p.A32A were novel and not found in the 192 controls and the 1000 Genomes Database (<http://browser.1000genomes.org>). The p.L402F mutation was predicted to be deleterious by SIFT software. The frequency of *COQ2* mutation in our cohort of patients was consistent with the previous studies (Table 1).

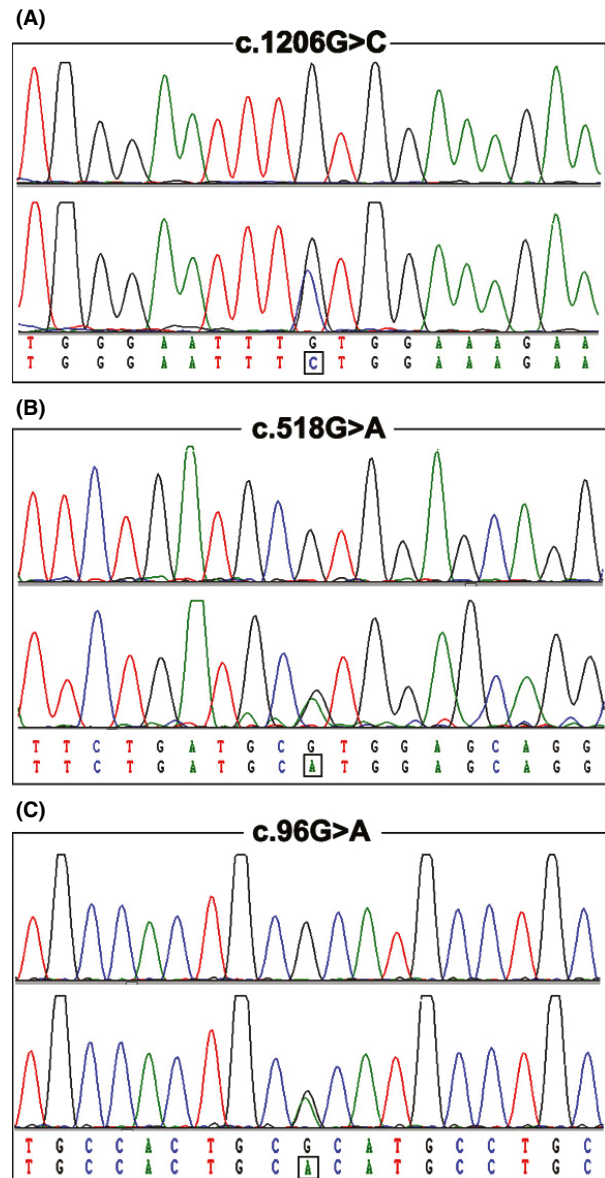


Figure 1 Mutation analysis of 3 *COQ2* mutations. Three mutations including c. 1206G>C (p.L402F), c. 518G>A (p.R173H), and c. 96G>A (p.A32A) were confirmed by direct sequencing. The upper panel for each chromatogram depicts the wild-type sequence, whereas the lower panel represents heterozygous mutated sequence.

Table 1 Frequencies of COQ2 variants in different cohorts

Population	Multiple system atrophy			Control		
	Carrier	Total	Frequency, %	Carrier	Total	Frequency, %
Japanese	4	363	1.10	2	520	0.38
European	4	223	1.79	0	315	0.00
North American	1	172	0.58	1	294	0.34
Korean	2	299	0.67	0	365	0.00
European-GER	0	788	0.00	1	600	0.17
European-UK	3	299	1.00	1	261	0.38
American	2	155	1.29	0	500	0.00
Chinese-southwest	4	312	1.28	0	598	0.00
Chinese-southeast	2	116	1.72	0	192	0.00

The variant c. 1178T>C (p.V393A) (referred to by Mitsui et al. [12] as V343A) was found in our cohort (Figure S1A). The frequency of C allele was 2.2% (5/232) in MSA cases and 0.5% (2/384) in controls, respectively ($P = 0.108$). We also found another variant c. 73T>G (p.L25V, rs150145464) (Figure 1B). The minor allele frequency (MAF) of G allele was reported to be 0.0046/10 by 1000 Genomes Project (National Center for Biotechnology Information). The frequency of G allele was 3.0% (7/232) in MSA cases and 2.9% (11/384) in controls, respectively ($P = 1.000$). Both of the results suggested that these two variants were polymorphisms.

Clinical Presentations of the Patients Carrying Missense Mutations

The patient harboring p.R173H mutation was a 59-year-old man, who had gait ataxia and dysarthria at age 55 years. In the following one year, he developed a difficulty in handwriting. Three years after the onset, he experienced orthostatic hypotension and urinary frequency. He had no family history of neurological diseases or movement disorders. On physical examination at 58 years old, he showed severe gait disturbance, dysmetria, dysarthria, bradykinesia, and hyperreflexia in all his extremities. There were no

muscular atrophy, sensory disturbance, and limitation of ocular movement. Brain MRI showed severe cerebellar and pontine atrophy (Figure 2). The polysomnography revealed an increase in arousal index.

The case carrying the p.L402F mutation had 4 years history of gait ataxia and dysarthria. When he was 54 years old, he began to exhibit gait unsteadiness, which made him fall to the ground sometimes. In addition, he complained of speech difficulty at the onset. One and a half years later, he suffered from urinary incontinence, constipation, and orthostatic lightheadedness. Neurological examinations at the age 55 revealed a broad-based ataxic gait, dysarthria, and mild bradykinesia. His muscle strength and tension was normal, but he was diffusely hyperreflexic with extensor plantar responses. Cranial nerve examination was unremarkable. His cognition was normal, based on a MMSE score of 28/30. Brain MRI demonstrated brain atrophy, predominantly cerebellar atrophy.

Discussion

Multiple system atrophy is generally regarded as a nongenetic disorder, because familial aggregation of MSA cases is rarely documented [8,9]. However, emerging evidence suggests that genetic

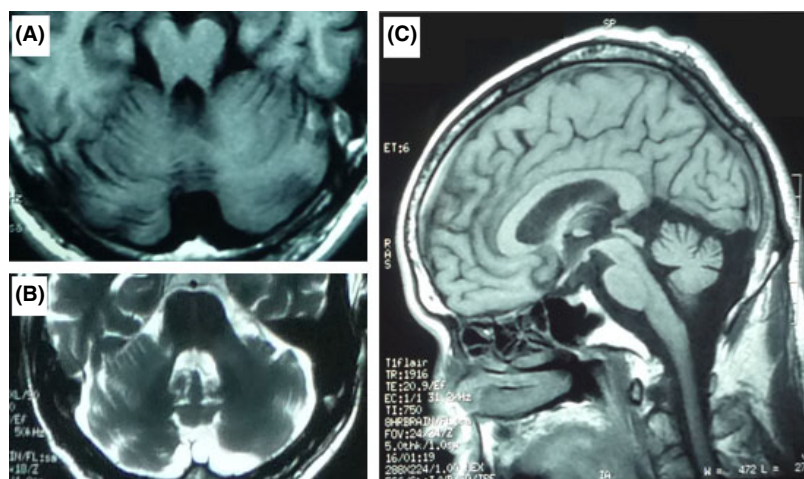


Figure 2 Representative brain MRI of the patient carrying c.518G>A (p.R173H). (A) T1-weighted image showing atrophy of the cerebellum and pons. (B) T2-weighted image showing atrophy of the cerebellum and pons with cross sign. (C) T1-weighted image showing atrophy of the cerebellum and pons.

variants increase the susceptibility of MSA. In 2009, Scholz and colleagues demonstrated that variants within *SNCA* were associated with an increased risk for MSA in Caucasian populations [11], which was subsequently replicated by other studies [10,18]. In addition to *SNCA* gene, variants within *COQ2* were recently identified to increase the risk of familial and sporadic MSA [12]. The *COQ2* gene was subsequently screened in MSA patients from South Korea, UK, Germany, and southwest China [13–17]. *COQ2* encoding Coq2 is essential in the biosynthetic pathway of coenzyme Q10 [19]. Coenzyme Q10 physiologically functions as electron carrier and participates in the mitochondrial respiratory chain. The deficiency of coenzyme Q10 results in the impairment of mitochondrial respiratory chain and oxidative injury, which was involved in the pathogenesis of MSA.

In the current study, we screened *COQ2* variants in 116 sporadic MSA-C cases, identifying 2 missense mutations (p.R173H and p.L402F) and a synonymous mutation (p.A32A). The p.R173H was recently documented in two cases (one MSA-P and one MSA-C) in a cohort of southwest Chinese patients with MSA [17], suggesting that it is common in the Chinese population and may play a distinct role in Chinese MSA patients. The p.L402F and p.A32A variants were novel and not reported previously. All of these three variants were not present in 192 healthy control individuals. Although we did not perform functional experiment to confirm the pathogenicity of p.R173H and p.L402F, both of them were predicted to be deleterious by the prediction software.

In addition, we identified 5 genetic variants in our MSA cases and control individuals. The p.V393A variant associated with MSA in the Japanese population was identified in 5/116 MSA cases and 2/192 controls in our study. The frequency of p.V393A

allele in our MSA cases (2.2%) is quite similar to that of a previous Chinese study (2.1% of MSA cases) [17]. This implies that the MAF of p.V393A may be approximately 2% in Chinese MSA cases. As shown in Table S3, the association of p.V393A variant with MSA was not significant in our cohort and the study from southwest China [17]. In the Korean study, the p.V393A was not associated with MSA either (MAF, 2.7% of cases, 2.6% of controls) [13]. Moreover, the p.V393A was not detected in Caucasian MSA cases [12,14–16]. Thus, p.V393A should be considered as a population-specific risk factor of Japanese patients with MSA.

Taking all the recent studies together, 24 of 2843 patients with MSA and 5 of 3645 health controls carried *COQ2* variants (Table S4). To date, 23 *COQ2* variants (Figure 3) have been identified with no hotspot in any population. Overall, the frequency of *COQ2* variants in MSA cases varied from 0% to 1.79% in different cohorts (Table 1), and the *COQ2* variants are more common in Asian cases than in Caucasian patients (Table S4), indicating a potential population-specific role of *COQ2* in MSA cases. This could be due to the different subtypes of MSA in Asia and Europe, as more *COQ2* variants found in MSA-C than MSA-P [12–17].

In summary, we identified 2 missense *COQ2* mutations (p.L402F and p.R173H) and one synonymous mutation (p.A32A) in a cohort of 116 Chinese MSA-C cases. The frequency of *COQ2* mutations (1.72%) was consistent with that of recent studies and demonstrated that *COQ2* tended to play a population-specific and subtype-dependent role in conferring susceptibility to MSA. Owing to the low frequency of *COQ2* variants identified in all the MSA cases reported, additional studies will be required to elucidate other genetic factors playing in the pathogenesis of MSA.

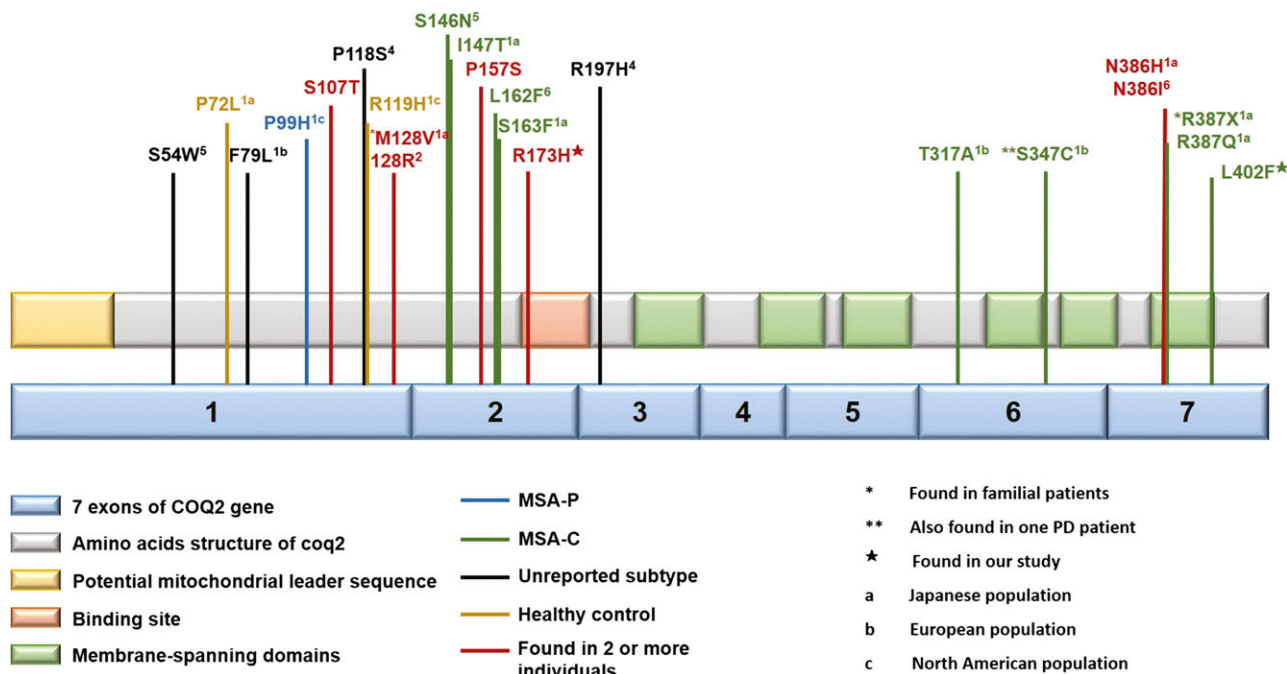


Figure 3 Schematic structure of Coq2 and distribution of identified *COQ2* variants. The *COQ2* gene consists of 7 coding exons. The coq2 protein is predicted to consist of a mitochondrial leader sequence, an allylic polyprenyl diphosphate substrate-binding site and 6 membrane-spanning domains. The p.R173 was located in the binding site and p.L402 in the transmembrane region. All the *COQ2* variants reported recently are shown.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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Supporting Information

The following supplementary material is available for this article:

Figure S1. Chromatograms of two variants including c.1178T>C (p.V393A) and c.73T>G (p.L25V) within COQ2. The upper panel for each chromatogram depicts the wild-type sequence, whereas the lower panel represents the heterozygous variant.

Table S1. Details of clinical manifestations of 116 MSA-C patients.

Table S2. PCR forward (F) and reverse (R) primers and annealing temperature designed for COQ2.

Table S3. Genotype and allele frequency of p.V393A allele in different populations.

Table S4. COQ2 variants in MSA cases and controls.