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Investigating *ELOVL7* coding variants in Multiple System Atrophy

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Abstract

Multiple system atrophy (MSA) is a rare sporadic, progressive parkinsonism characterised by autonomic dysfunction. A recent genome-wide association study reported an association at the Elongation of Very Long Fatty Acids Protein 7 (*ELOVL7*) locus with MSA risk. Four independent and unrelated cohorts were assessed, consisting of pathologically confirmed MSA cases, Parkinson's disease (PD) cases, and two unrelated, healthy control groups. All exons of *ELOVL7* were sequenced in pathologically confirmed MSA cases; data for PPMI samples and Biobank

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controls was extracted from whole genome sequence. Coding variants in *ELOVL7* were extremely rare, and we observed no significant association of *ELOVL7* coding variants with risk of MSA.

Keywords

Multiple system atrophy; synucleinopathy; genetics; lipids; ELOVL7

1. Introduction

Multiple system atrophy (MSA) is a rare and progressive neurodegenerative movement disorder, characterized by autonomic dysfunction and the accumulation of pathological alpha-synuclein (aSyn) aggregates in the oligodendroglia. MSA is clinically categorized into two subtypes: MSA-Parkinsonism (MSA-P), characterized by levodopa-unresponsive parkinsonism (i.e., bradykinesia and rigidity) and MSA-cerebellar (MSA-C), presenting with cerebellar ataxia. Neurodegeneration is typically seen in both the striatonigral and olivopontocerebellar systems. MSA-P is characterized by predominant striatonigral degeneration (SND), while MSA-C is characterized by predominant olivopontocerebellar atrophy (OPCA). Patients in which both striatonigral and olivopontocerebellar systems are equally affected is categorized as MSA-mixed cases. [1, 2].

MSA is predominantly sporadic with only a handful of familial cases reported [3]. As a result, the genetic etiology of MSA is not well defined. The only MSA genome-wide association study (GWAS) to date was performed in 2016 as a global consortium effort. Due to the rare nature of MSA, the study comprised of 918 MSA cases and 3,864 controls, which is relatively small for GWAS designs [4]. Likely due to low statistical power, no genetic loci reached genome-wide significance; however, four variants were highlighted as ‘of interest’ ($P<1 \times 10^{-6}$). One of these SNPs was an intronic variant (rs7715147) located in Elongation of Very Long Fatty Acids Protein 7 (*ELOVL7*). Interestingly, variants in the *ELOVL7* loci also reached significance in GWAS meta-analyses of Parkinson’s disease (PD) [5, 6] and mutations in *ELOVL4* (SCA34) [7] and *ELOVL5* (SCA38) [8] are a cause of autosomal dominant spinocerebellar ataxias (SCA) - which are disorders that have cerebellar degeneration in common with MSA [9].

ELOVL7 belongs to a family of seven elongases which play a critical role in the synthesis of very long chain fatty acid (VLCFA) [10] . ELOVL7 elongates both saturated and monosaturated fatty acids and assist in the formation of lipids [11, 12]. The brain is the most lipid affluent organ in the body; the majority of the tissue constitutes myelin sheaths, which are composed of lipids and cholesterol. Oligodendrocytes, the cells most vulnerable to aSyn aggregates in MSA, may be particularly sensitive to lipid dyshomeostasis [13].

Considering the important biological function of *ELOVL7*, its genetic association in MSA (and PD) and the links between *ELOVL4* and *ELOVL5* and SCA, we investigated the role of coding variation in *ELOVL7* in synucleinopathy.

2. Materials and Methods

2.1 Study Design

A total of 167 pathologically-confirmed MSA cases were ascertained by the Mayo Clinic Brain Bank for Neurodegenerative Disorders between 1998 and 2015 and were neuropathologically evaluated by one neuropathologist (DWD). Among these, 64 cases were included in a previous GWAS report [4]. Neuropathological diagnosis of MSA was based on α-synuclein immunohistochemistry (NACP, 1:3000 rabbit polyclonal, Mayo Clinic antibody, FL) according to the established criteria [14]. Pathological subtype of MSA was based on the severity of neurodegeneration of vulnerable brain regions. MSA-P had more severe pathology in the striatonigral system; MSA-C had more severe pathology in the olivopontocerebellar system; and MSA-mixed had equally severe pathology in both systems [15].

To assess the role of *ELOVL7* variants in PD, we utilized whole-genome sequence (WGS) data from 396 idiopathic PD patients and 183 controls from the Parkinson's Progression Markers Initiative (PPMI) [16]. Inclusion and exclusion criteria for the PPMI cohorts are described in the PPMI portal (https://www.ppmi-info.org). A clinical control series of 834 subjects without neurodegenerative disease from the Mayo Clinic Biobank with WGS data was included [17]. All subjects were Caucasian, non-Hispanic, and unrelated. Characteristics of patients and controls are displayed in Table 1. This study was approved by the Mayo Clinic Institutional Review Board and patient/next-of-kin provided signed consent for the research study. The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

2.2 Genetic Analysis

Sanger Sequencing and Copy Number Assessment—Primers were designed for non-coding exon 3, where the rs7715147 SNP resides [4], and coding exons 5-11 of *ELOVL7* (NM_024930) . NM_024930 codes for 11 exons in total, with seven coding exons from 5-11. Bidirectional Sanger sequencing for exons 3 and 5-11 was performed in all 167 pathologically confirmed MSA cases on an ABI 3730xl DNA Analyzer (Applied Biosystems, CA, USA). Sequences were aligned and annotated using Seqscape software (v3.0) (Thermo Fisher, MA, USA). Call rates were >98% for all variants and there was no evidence of a departure from Hardy Weinberg Equilibrium (all P>0.05). Copy number variation (CNV) of *ELOVL7* was assessed in 167 pathologically-confirmed MSA cases using a commercially available TaqMan™ Copy Number Assay probe (Hs01818937_cn; ThermoFisher Scientific, MA, USA). PCRs were performed on a Quant Studio™ 7 Flex Real-Time PCR System (ThermoFisher Scientific, MA, USA) according to the manufacturer's instructions. An RNaseP TaqMan™ Copy Number Reference Assay probe (catalog number: 4403326) was used as an endogenous control to normalise differences in DNA concentration (ThermoFisher Scientific, MA, USA).

Whole Genome Sequencing in PPMI series and Mayo Clinic Biobank controls —The PPMI series and Mayo Clinic Biobank control WGS data were processed using the

Mayo Genome GPS v4.0 pipeline. Functional annotations of variants were performed using ANNOVAR (version 2016Feb01). Genotype calls with genotype quality (GQ) < 10 and/or read depth (DP) < 10 were set to missing, and variants with an Edit Distance (ED) > 4 were removed from all subsequent analyses. For all analyses, only variants that passed Variant Quality Score Recalibration (VQSR) and with a call rate > 95% were considered, unless otherwise specified. The transition/transversion ratio for this final variant call set is 2.04. All variants in *ELOVL7* were extracted using SNP & Variation Suite v8.8.3 (Golden Helix Inc., MT, USA). Data for all exons was captured with an average coverage of the coding exons of *ELOVL7* in the PPMI series of 38.8X (minimum coverage of 37.0X) and Mayo Clinic Biobank controls with an average 39.6X (minimum coverage of 38.2X).

Statistical analysis—Given the rare nature of the *ELOVL7* variants that were identified, single-variant comparisons vs. controls for PD and MSA patients were not performed due to the very low power such comparisons would have to detect a difference. Instead, we performed gene-burden tests, comparing the frequency of presence of the minor allele for any *ELOVL7* variant vs. controls separately for PD patients and MSA patients using Fisher's exact test. P-values <0.05 were considered as statistically significant, and all statistical tests were two-sided. Statistical analyses were performed using R Statistical Software (version 3.6.2).

3. Results

All exonic variants that were observed in any series were rare (MAF<1%). Two variants were observed only in pathologically confirmed MSA cases (p.S33S in one MSA-C case and p.H150Y in one MSA-mixed case) and six rare coding variants (MAF<1%) were observed in either one or two Biobank controls (Table 2). To further assess if CNV in *ELOVL7* was influencing MSA risk, we investigated CNV at exon 7 in all pathologically confirmed MSA samples. All cases reported normal relative fluorescent unit (RFU) ranges (0.8–1.2 RFU) at *ELOVL7* exon 7.

To explore the genetic overlap between pathophysiologically similar diseases, WGS from PPMI patients with PD and controls was compared to WGS from Mayo Clinic Biobank controls. Three different rare coding variants were identified in *ELOVL7* in patients with PD (p.D20G and p.S79Y) and controls (p.S79Y and p.R106W) from the PPMI series (Table 2). In total, only four different exonic variants were observed in either the pathologically confirmed MSA or PD cases. Two variants (p.S33S and p.H150Y) were only observed in MSA patients and not in controls. When examining the presence of the minor allele of any exonic variant, no significant difference was noted between the combined PPMI/Biobank control group and either MSA patients (1.0% vs. 1.2%, P=0.68) or PD patients (1.0% vs. 0.8%, P=1.00).

4. Discussion

Variants in *ELOVL7* have been nominated in genome-wide association studies of PD and MSA (P=2.5E-23 and P=2.9E-07 respectively) [4-6]. The rs7715147 variant which was identified by Sailer *et al.* in 2016 in the MSA GWAS [4], was different from the *ELOVL7*

SNP, rs1867598, which reached genome-wide significance in PD [6]; these two SNPs appear to tag independent signals (r^2=0.03) [18]. Whilst we were interested in coding variation in *ELOVL7* in MSA, we felt it important to also assess possible linkage disequilibrium with the non-coding GWAS SNP [4], and therefore, we sequenced non-coding exon 3, where rs7715147 resides.

Our assessments of Sanger sequencing and WGS in MSA and PD cohorts respectively reported no statistically significant differences in the frequencies of rare variants (MAF<1%) in *ELOVL7* when compared to WGS data from controls; we observed only a few carriers of exonic variants in individual disease cohorts. Interestingly, p.S33S and p.H150Y were only observed in MSA patients and not in either of the control groups or PD cohort. However, as numbers reported are very small, it is difficult to interpret a relationship between the variants and disease risk and pathology. For a genetic study, our sample sizes in this study were small and power to detect associations was extremely limited. Furthermore, different sequencing methods were employed across our cohorts that may have limitations when comparing frequency of variants across platforms. However, Sanger sequencing and WGS data are both robust and thorough sequencing methods which provide accurate and reliable sequencing data. These technologies have been, and are, continually used as the gold standard for genetic sequencing and exploratory studies [19] therefore they are suitable and comparable techniques to use in this work. Our WGS analysis showed no significant difference in the coverage of the *ELOVL7* exons between the PPMI series and the Mayo Clinic Biobank control data with an overall average of 37.5X per exon.

This is the first study to assess exonic genetic variation in *ELOVL7* in cohorts of pathologically defined MSA which have been compared to two independent control cohorts, as well as a series of patients with PD. Despite not observing presence of, or increased burden of, rare coding variants in MSA or PD cases, this study alone does not exclude the possibility of ELOVL7 driving α-synucleinopathy. This study further highlights the need for ongoing independent genetic assessments to explore regions from GWAS in more detail in diseased cohorts, to validate their role in disease pathology. Although outside the scope of this present study, conducting future studies in both non-coding and regulatory regions of *ELOVL7* in MSA will be important to understand the influences of variant load on disease pathology and risk.

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Highlights

- Genome-wide association studies have implicated *ELOVL7* variation in MSA risk
- Exonic screening of coding variants in MSA and control subjects for coding variants
- Coding variants in *ELOVL7* are extremely rare, highlighting its biological importance
- *ELOVL7* coding variants or copy number mutation is not driving MSA risk

Table 1:

Cohort demographics and DNA information.

	Pathologically confirmed MSA cases (N=167)	PPMI PD (N=396)	PPMI Controls (N=183)	Biobank Controls (N=834)
Age[1]	66 (47, 91)	61 (33, 85)	70 (40, 93)	61 (31, 96)
Sex (male)	96 (57.5%)	255 (64.4%)	117 (63.9%)	426 (51.1%)
MSA subtype				
MSA-C	27 (16.2%)	--	--	--
MSA-P	73 (43.7%)	--	--	--
MSA-mixed	66 (39.5%)	--	--	--
MSA-unclassified[2]	1 (0.6%)	--	--	--
Race				
Caucasian	167 (100.0%)	375 (94.7%)	172 (93.9%)	834 (100.0%)
Ethnicity				
Non-Hispanic or Latino	167 (100%)	388 (97.9%)	181 (98.9%)	834 (100.0%)
DNA Source	Cerebellum	Whole blood	Whole blood	Whole blood
Genetic data source	Sanger sequencing	Whole genome sequencing	Whole genome sequencing	Whole genome sequencing

[1]The sample median (minimum, maximum) is given for age. The age of death is provided for pathologically confirmed MSA patients, Age at onset is given for PPMI PD patients and current age is given for PPMI controls.

[2]Only the pons was available for histologic assessment for one MSA patient, therefore they were considered ‘MSA-unclassified’ as they could not be diagnosed as either MSA-P or MSA-C.

Table 2:

Frequencies of rare *ELOVL7* variants from sequencing and genotyping analysis.

				No. (%) of carriers of the minor allele				
rsID	**Amino acid**	**Major allele**	**Minor allele**	**Pathologically confirmed MSA cases (N=167)**	**PPMI PD patients (N=396)**	**PPMI Controls (N=183)**	**Biobank controls (N=834)**	**gnomAD WGS/WEs allele frequency (N~120-140K)**
rs144954272	D20G	T	C	0	2 (0.5%)	0	2 (0.2%)	0.001
rs150312172	S33S	G	A	1 (0.6%)	0	0	0	0.0001
rs147783451	M56V	T	C	0	0	0	1 (0.1%)	0.0001
rs372933045	F78L	A	G	0	0	0	1 (0.1%)	0.0002
rs368309905	S79Y	G	T	0	1 (0.3%)	1 (0.5%)	2 (0.2%)	0.0001
rs749088345	R106W	C	T	0	0	1 (0.5%)	0	0.0001
rs200379193	R137C	G	A	0	0	0	1 (0.1%)	0.00004
rs1181616389	H150Y	C	T	1 (0.6%)	0	0	0	0.00001
rs757660472	A165V	G	A	0	0	0	1 (0.1%)	0.00001
Presence of the minor allele for any variant				2 (1.2%)	3 (0.8%)	2 (1.1%)	8 (1.0%)	NA

Sequencing revealed rare *ELOVL7* variants in pathological MSA cases, PPMI patients with PD and controls, and Biobank controls. GnomAD v3.1 frequencies are from Ensembl transcript ID ENST00000508821.6 (NA= not applicable) from whole-genome sequence (WGS) or whole-exome sequence (WES) data.