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SHORT REPORT

VPS35 and DNAJC13 disease-causing variants in essential tremor

Alex Rajput[1,5], Jay P Ross[2,5], Cecily Q Bernales[2], Sruti Rayaprolu[3], Alexandra I Soto-Ortolaza[3], Owen A Ross[3], Jay van Gerpen[4], Ryan J Uitti[4], Zbigniew K Wszolek[4], Ali H Rajput[1] and Carles Vilariño-Güell*[,2]

Exome-sequencing analyses have identified vacuolar protein sorting 35 homolog (*VPS35*) and DnaJ (Hsp40) homolog, subfamily C, member 13 (*DNAJC13*) harboring disease-causing variants for Parkinson disease (PD). Owing to the suggested clinical, pathological and genetic overlap between PD and essential tremor (ET) we assessed the presence of two *VPS35* and *DNAJC13* disease-causing variants in ET patients. TaqMan probes were used to genotype VPS35 c.1858G>A (p.(D620N)) (rs188286943) and DNAJC13 c.2564A>G (p.(N855S)) (rs387907571) in 571 ET patients of European descent, and microsatellite markers were used to define the disease haplotype in variant carriers. Genotyping of *DNAJC13* identified two ET patients harboring the c.2564A>G (p.(N855S)) variant previously identified in PD patients. Both patients appear to share the disease haplotype previously reported. ET patients with the VPS35 c.1858G>A (p.(D620N)) variants were not observed. Although a genetic link between PD and ET has been suggested, DNAJC13 c.2564A>G (p.(N855S)) represents the first disease-causing variant identified in both, and suggests the regulation of clathrin dynamics and endosomal trafficking in the pathophysiology of a subset of ET patients.
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INTRODUCTION

Essential tremor (ET) and Parkinson disease (PD) are the two most common movement disorders in adults, ET affecting up to 6% of the general population and PD ~1% of the population at the age of 65 years.[1,2] Although ET and PD are considered distinct entities, they both present tremor as a common clinical feature, and a pathological and genetic overlap has been suggested.[3,4] Our exome-sequencing analyses in multi-incident families with PD have identified two novel genes,—vacuolar protein sorting 35 homolog (*VPS35*) and DnaJ (Hsp40) homolog, subfamily C, member 13 (*DNAJC13*)— harboring disease-causing variants.[2,5,6] Both genes encode essential proteins for clathrin-mediated endocytosis and endosomal recycling of membrane proteins.[7] Although several variants have been described in each gene, disease causality has only been confirmed for VPS35 c.1858G>A (p.(D620N)) (rs188286943) and DNAJC13 c.2564A>G (p.(N855S)) (rs387907571).

Given the potential overlap between ET and PD, we evaluated the incidence of VPS35 c.1858G>A (p.(D620N)) and DNAJC13 c.2564A>G (p.(N855S)) in a series of patients with ET from North America.

METHODS

We included 571 ET patients of European descent from North America. Average patient age was 68.5 ± 12.0 years, with an average age of onset of 51.8 ± 19.2 years and a male to female ratio of 1:1.22. All patients were examined and observed longitudinally by a movement disorder neurologist and diagnosed according to standard criteria.[8] The ethical review board of each institution approved the study and all participants provided informed consent.

TaqMan probes were used to genotype VPS35 c.1858G>A (p.(D620N)) (NM_018206.4) (ClinVar accession: SCV000044406) and DNAJC13 c.2564A>G (p.(N855S)) (NM_015268.3) (ClinVar accession: SCV000082642 and SCV000172129) on an ABI 7900 real-time PCR system and they were analyzed with SDS 2.4 software (Life Technologies, Carlsbad, CA, USA). Five-microliter reactions consisting of 20 ng of DNA, 1x TaqMan genotyping master mix and 1x TaqMan SNP genotyping assay (*VPS35*, AHMSMQ4; *DNAJC13*, AHS1CLB) with appropriate genomic controls were used to genotype both variants. Thermal cycling conditions consisted of one enzyme activation step at 95 °C for 10 min followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min for VPS35 c.1858G>A (p.(D620N)) or 50 cycles at 95 °C for 15 seconds and 62 °C for 1 min for DNAJC13 c.2564A>G (p.(N855S)). All heterozygous genotypes were confirmed by Sanger sequencing as previously described.[9] Microsatellite markers spanning the *DNAJC13* locus were run on an ABI 3730xl and analyzed using GeneMapper 4.0 (Life Technologies) to define the disease haplotype, as previously described.[5]

RESULTS

Genotyping of DNAJC13 c.2564A>G (p.(N855S)) in ET patients identified two heterozygous carriers (ET1 and ET2). ET1 is a female patient with a family history of ET on her maternal side. At the age of 69 she developed right hand tremor followed by left hand tremor 2 years later. On the last examination at the age of 73, she presented horizontal head tremor, vocal cord tremor on phonation and kinetic tremor in the upper limbs. Neither resting tremor, bradykinesia nor rigidity were observed. ET2 is a male patient with an autosomal dominant family history of disease, whose mother and one sister have

[1]Division of Neurology, University of Saskatchewan and Saskatoon Health Region, Saskatoon, SK, Canada; [2]Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada; [3]Department of Neuroscience, Mayo Clinic, Jacksonville, FL, USA; [4]Department of Neurology, Mayo Clinic, Jacksonville, FL, USA
*Correspondence: Professor C Vilariño-Güell, Department of Medical Genetics, University of British Columbia, 5639 - 2215 Wesbrook Mall, Vancouver, BC V6T 2B5, Canada. Tel: +1 604 827 1303; Fax: +1 604 822 7299; E-mail: carles@can.ubc.ca
[5]These authors contributed equally to this work.
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also been diagnosed with ET. He developed right upper limb tremor at the age of 61 and left upper limb tremor at the age of 63. After 5 years of disease, he presented action tremor and occasional resting tremor in both upper limbs. Neither patient presented additional neurological symptoms or required medication for their tremor. No additional affected family members were available for segregation analysis.

DNAJC13 c.2564A>G (p.(N855S)) haplotype analysis suggests that both patients share the previously described disease haplotype (Table 1), although Dutch–German–Russian Mennonite ancestry was not reported.[5] Since the haplotype phase for ET2 could not be resolved, and only marker alleles downstream of *DNAJC13* appears to be shared, it is also possible that this patient presents a novel disease haplotype.

The VPS35 c.1858G>A (p.(D620N)) variant was not observed in any of the ET patients characterized in this study.

Table 1 Chromosome 3q21.3-q22.2 haplotype for DNAJC13 p.(N855S) carriers

Marker	*Position*	*SK1*	*ET1*	*ET2*	*CEPH frequency*
D3S3606	127 200 206	**173**	**173**	171/173	0.19
D3S3607	127 272 891	**144**	**144**	154/156	0.11
D3S1587	130 798 818	**215**	**215**	215/219	0.09
D3S3514	131 580 046	**260**	**260**	258	0.05
D3S1292	131 630 309	**154**	**154**	144	0.05
DNAJC13-N855S	132 196 839	**G**	**G**	**G**/A	
D3S1273	132 826 238	**118**	**118**	**118**/112	0.11
D3S1290	132 990 918	**222**	**222**	**222**/210	0.06
D3S3657	133 425 958	**261**	**261**	**261**/265	0.34
D3S3684	133 923 266	**164**	**164**	**164**/170	0.32

Markers are shown with their physical locations (NCBI Build 37.1) and with allele sizes consistent with Centre d'Etude du Polymorphisme Humain (CEPH) standards. Previously reported disease haplotype (SK1) with the corresponding allele frequency from the CEPH database is provided as a reference. Shared alleles between SK1 and ET1 or ET2 are indicated in bold. For markers with an unknown phase both alleles are given.

DISCUSSION

Genotyping of VPS35 c.1858G>A (p.(D620N)) and DNAJC13 c.2564A>G (p.(N855S)) resulted in the identification of two ET patients harboring the *DNAJC13* variant, resulting in a carrier frequency of 0.3%. Although we did not genotype control subjects, it should be noted that we had previously genotyped over 2600 unrelated healthy individuals, including 117 individuals of reported Mennonite ancestry, without observing either variant.[2,5]

The identification of the DNAJC13 c.2564A>G (p.(N855S)) variants in two patients diagnosed with ET provides additional support for a common mechanism of disease between PD and ET, at least among carriers of this variant. Although a genetic link between these two neurological diseases has been previously suggested,[1] DNAJC13 c.2564A>G (p.(N855S)) represents the first disease-causing variant identified in both diseases, and suggests a dysregulation of clathrin dynamics and endosomal trafficking as the cause of the disease in a subset of ET patients, or an increased susceptibility to develop neurological diseases.

Both VPS35 and DNAJC13 are involved in the endosomal-trafficking network and have been found to co-localize in neurons.[5,7] Although the VPS35 c.1858G>A (p.(D620N)) variant was not identified in our ET series, it is still possible that disease-causing variants in *VPS35* result in the dysregulation of endosomal compartmentalization and trafficking observed in cells transfected with DNAJC13 c.2564A>G (p.(N855S)),[5] and the onset of ET. Alternatively, VPS35 c.1858G>A (p.(D620N)) may be found only in patients with PD. Disease-causing variants in *VPS35*, encoding a critical component of the retromer cargo-recognition complex,[10,11] could result in specific membrane receptor deficiencies causing the onset of PD, whereas disease-causing variants in *DNAJC13*, which regulates the upstream dynamics of clathrin coats on early endosomes, are likely to result in a broader biological outcome and disease phenotype.[7]

To further define the prevalence of *DNAJC13* and *VPS35* disease-causing variants in patients diagnosed with movement disorders, as well as elucidate the role of the endosomal-trafficking network in neurodegeneration, it is essential to evaluate the presence of these variants not only in additional PD and ET patients, but also in other neurodegenerative diseases.

CONFLICT OF INTEREST

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