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Target-enriched sequencing of chromosome 17q21.31 in sporadic tauopathies reveals no candidate variants

Cristina Razquin^a, Sara Ortega-Cubero^{b,c,d}, Estefania Rojo-Bustamante^{c,e}, Monica Diez-Fairen^f, Elena Lorenzo^{c,d}, Elena Alonso^{c,d}, Mario Ezquerra^{d,g}, Owen A. Ross^{h,i}, Maria Carcel^f, Oswaldo Lorenzo-Betancor^{c,j}, Alexandra I. Soto^h, Jeremy D. Burgess^h, Nilüfer Ertekin-Taner^{i,k}, Dennis W. Dickson^h, Maria A. Pastor^{d,l}, Eduard Tolosa^{d,g}, Pau Pastor^{c,d,f,*}

^aDepartment of Preventive Medicine and Public Health, University of Navarra, Pamplona, Spain; Instituto de Investigación Sanitaria de Navarra, Pamplona, Spain; CIBER Fisiopatología de la Obesidad y Nutrición (CIBERObn), Instituto de Salud Carlos III, Madrid, Spain

^bDepartment of Neurology and Neurosurgery, Hospital Universitario de Burgos, Burgos, Spain

^cNeurogenetics Laboratory, Division of Neurosciences, Center for Applied Medical Research, University of Navarra (CIMA), Pamplona, Spain

^dCentro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas, CIBERNED, Instituto de Salud Carlos III, Madrid, Spain

^eDepartment of Biochemistry and Genetics, School of Science and Neuroprotective Strategies Laboratory, Division of Neurosciences, Center for Applied Medical Research (CIMA), University of Navarra, Pamplona, Spain

^fMovement Disorders Unit, Department of Neurology, Hospital Universitari Mutua de Terrassa, and Fundació per la Recerca Biomèdica i Social Mútua Terrassa, Terrassa, Barcelona, Spain

^gParkinson's Disease and Movement Disorders Unit, Neurology Service and Institut Clínic de Neurociències, Hospital Clínic de Barcelona, Universitat de Barcelona, IDIBAPS, Barcelona, Spain

^hDepartment of Neuroscience, Mayo Clinic, Jacksonville, FL, USA

ⁱDepartment of Clinical Genomics, Mayo Clinic, Jacksonville, FL, USA

^jVeterans Affairs Puget Sound Health Care System, and Department of Neurology, University of Washington School of Medicine, Seattle, WA, USA

^kDepartment of Neurology, Mayo Clinic, Jacksonville, FL, USA

*Corresponding author at: Department of Neurology, University Hospital Mutua, de Terrassa, Pl. Dr. Robert, 5, 08221-Terrassa, Barcelona, Spain. Tel.: +34 937365050; fax: +34 937365059. pastorpau@gmail.com (P. Pastor).

Authors' contribution: Conception and design of the study were performed by CR and PP. Acquisition and analysis of clinical data were by PP, MAP, and SO-C. Acquisition of genetic data was by EL, EA, CR, SO-C, and ER-B. Analysis of genetic data was by CR, PP and SOC. The manuscript was written by CR and PP. All the authors revised the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.neurobiolaging.2017.12.026>.

Disclosure statement

The authors have no actual or potential conflicts of interest.

¹Neuroimaging Laboratory, Division of Neurosciences, Center for Applied Medical Research, University of Navarra (CIMA), Pamplona, Spain

Abstract

The main genetic risk factors for progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) are located at chromosome 17q21.31. The identification of risk H1 subhaplotypes suggests that disease-specific variants can be identified by resequencing the 17q21.31 region (1.4 Mb) in carriers of risk H1 subhaplotypes. We hypothesized that PSP/CBD H1 subhaplotype carriers could have undergone a mutational event absent among unaffected carriers leading to the disease risk. We performed this strategy in definite PSP subjects, definite CBD subjects, and healthy controls and tried to replicate the findings in a larger PSP/CBD case-control series. In the resequencing process, 40 candidate variants were identified, but an association between PSP and rs76970862 was replicated only using an unadjusted model. Gene expression association analysis of this variant suggested no potential functional effect. Although our results failed to identify disease-associated variants, it is still possible that the risk of PSP/CBD at chromosome 17 is driven by rare variants, even in PSP/CBD H1 cases or variants located outside the capture regions.

Keywords

Genetics; *MAPT*; Progressive supranuclear palsy

1. Introduction

Progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) are progressive neurodegenerative disorders characterized by abnormal deposition of microtubule-associated tau protein in the brain (Boeve et al., 2003). PSP and CBD are generally sporadic, though genetic variants may increase the disease risk. The main PSP/CBD disease risk *locus* is an ancestral large inversion at chromosome 17q31.21 (~900 kb) that depicts H1/H2 haplotypes in which the microtubule-associated protein tau (*MAPT*) gene is located (Baker et al., 1999; Hoglinger et al., 2011; Kouri et al., 2015; Pastor et al., 2002). We previously found several H1 subhaplotypes (mainly H1E'C and H1Q), which expand to *MAPT* and corticotropin-releasing hormone receptor 1 (*CRHR1*) genes that could contain PSP/CBD risk variants. We hypothesized that the disease variants are linked to H1 subhaplotypes and probably located either at *MAPT* gene, including flanking regulatory regions, or even at neighboring genes (Cruchaga et al., 2009). To address this question, we resequenced the chromosome 17q21.31 region (1.4 Mb) in carriers of risk H1 subhaplotypes with pathologically confirmed PSP, CBD, and healthy controls and attempted to replicate these findings in larger series of clinical or pathologically confirmed PSP and CBD cases.

2. Methods

Our study had 4 stages: in the first “sequencing sample” stage, deep next generation sequencing of the chromosome 17q21.31 region was performed in pathologically confirmed carriers of risk H1 subhaplotypes (Cruchaga et al., 2009) to identify candidate variants

according to a priori established genetic models (Supplementary Material). In the second stage “discovery sample”, the candidate variants identified were genotyped in a clinical Spanish series with probable PSP, CBD, and healthy controls ($n = 356$). The variants associated with PSP or CBD were further genotyped in the third stage in a US case-control series (“replication sample”) composed of controls and pathologically confirmed PSP and CBD ($n = 1807$). Finally, brain gene expression association analysis of potential candidate variants was performed (Supplementary Material).

3. Results

3.1. Sequencing sample

Deep sequencing of the targeted 17q21.31 region was performed in 24 subjects, including the entire sequence of the region including exons, introns, and intergenic regions. This sequencing sample was composed of definite PSP ($n = 11$; Hauw et al., 1994), CBD ($n = 3$), and neuropathologically normal controls ($n = 10$; 9 were H1/H1 and 1 H2/H2 to discard H1/H2 single-nucleotide polymorphisms for the analysis; Fig. 1). The number of risk H1 subhaplotypes was balanced between the cases and controls selected for deep sequencing (Supplementary Table 1). In fact, all the PSP and CBD subjects carried at least 1 or 2 of the risk subhaplotypes. However, among the PSP/CBD cases of the sequencing samples, there was an enrichment of subjects carrying the H1Q subhaplotype (Cruchaga et al., 2009), which has been more strongly associated with the disease. A mean coverage of 125 ± 79 fold in the targeted region was reached. Under a dominant model, we identified 40 variants and a recessive model showed no variants (Table 1). Thirty-four variants were single-nucleotide variations (31 were noncoding either intronic or intergenic) and 6 noncoding indels. Ten variants were discarded owing to reported minor allelic frequency >0.1 and other 3 because of genotyping failure.

3.2. Discovery sample

The 26 selected variants were further selected to be screened in the Spanish case-control clinical cohort (Table 2; controls $n = 124$, PSP $n = 161$, and CBD $n = 71$). Genotyping of 4 variants in the whole sample was discontinued because they showed the same frequency as the other variants, suggesting that they were in complete linkage disequilibrium (LD; Table 2). Three variants deviated from the Hardy-Weinberg equilibrium owing to genotyping errors and were discarded. Among the 19 variants fully genotyped, 3 LD blocks were seen (Supplementary Fig. 1). rs76970862 was associated ($p < 0.05$) with PSP and CBD, and the rs17763658 showed a trend for association ($p < 0.08$) with PSP and CBD in the Spanish discovery sample. The variant rs76970862 was located at the intron of *CRHR1* gene, and rs17763658 was located at the intergenic *CRHR1-SPPL2C* gene. rs76970862 was not in LD with the PSP-associated subhaplotypes H1E, H1Q (Cruchaga et al., 2009), and H1C (Pittman et al., 2005; Supplementary Fig. 2).

3.3. Replication sample

rs76970862 and rs17763658 were genotyped in a US sample composed of 803 pathologically confirmed PSP, 133 pathologically confirmed CBD, and 871 clinical controls ($n = 1807$; Table 1). The association between rs76970862 (A/G) and PSP was replicated in

the crude model (odds ratio = 1.40; 95% confidence interval: 1.03–1.92; $p = 0.033$ for the risk allele G).

3.4. Gene expression association analysis

Association analysis of rs76970862 and rs17763658 with brain expression levels of *CRHR1* or *MAPT* in the PSP cerebellum or temporal cortex showed no significant association (Supplementary Material).

4. Discussion

Our study aimed to depth-sequence the chromosomal 17q21.31 region to identify the main PSP/CBD risk variants. Further replication of the variants associated in the discovery sample showed that only the intronic variant (Chr 17 43,786,683; rs76970862 [A/G]) located at the *CRHR1* gene locus appeared to be associated with the risk for PSP and CBD. The gene expression analysis of the rs76970862 variant showed no effects on *CRHR1* or *MAPT* brain expression. Although the selection of the *sequencing sample* was performed according to H1 subhaplotypes to minimize variability, it is still possible that either the rs76970862 variant may be in LD with another variant having a functional effect or we failed to capture other variants because of the small initial sample size.

Another limitation of our study is that the restrictive criteria for selecting the candidate variants limit the practical number of variants for subsequent analysis, in the sense that we only could have identified variants contributing to the cause of at least 20% (3/14) of cases. Our assumption of finding high-frequency variants in cases contributing to risk may be one of the reasons of our negative findings. Another possibility is that the risk of PSP/CBD at chromosome 17 is driven by rare variants, even in PSP/CBD H1 cases (<20%) or variants located outside the capture regions (Allen et al., 2016; Kouri et al., 2015).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Flowchart of the study. Abbreviations: CBD, corticobasal degeneration; PSP, progressive supranuclear palsy.

Table 1

Demographics of the sequencing sample

	Sex (n (%) of males)	Age at onset (mean $\pm$ SD)	Age at death (mean $\pm$ SD)
Sequencing sample			
Control (n = 10)	2 (20)	NA	76.7 $\pm$ 4.8
PSP (n = 11)	7 (64)	65.4 $\pm$ 4.9	71.1 $\pm$ 4.7
CBD (n = 3)	1 (33)	67.3 $\pm$ 2.1	75.7 $\pm$ 7.1
Discovery sample			
Control (n = 124)	57 (46)	NA	NA
PSP (n = 161)	75 (47)	66.0 $\pm$ 7.5	NA
CBD (n = 71)	34 (48)	66.0 $\pm$ 9.6	NA
Replication sample			
Control (n = 871)	367 (42)	NA	65.1 $\pm$ 12.8
PSP (n = 803)	425 (53)	NA	74.8 $\pm$ 7.6
CBD (n = 133)	66 (50)	NA	70.1 $\pm$ 8.4

Key: CBD, corticobasal degeneration; NA, not available; PSP, progressive supranuclear palsy; SD, standard deviation.

Table 2

Genetic variants analyzed in the Spanish discovery sample

Chr:Position	ID dbSNP	Reference/ alternative allele	Gene	Control alternative allele freq	PSP alternative allele freq	p	CBD alternative allele freq	p	OR (95% CI) for control vs. PSP + CBD	p
17:43308705	NA	[G/-]	Intron FMNL-1	0.10	0.085	0.498	0.058	0.209	0.76 (0.441–1.331)	0.318
17:43333700	rs111535252	[G/A]	Intron NR_024434	0.1	0.064	0.174	0.068	0.36	0.62(0.323–1.220)	0.148
17:433335567	rs77661889	[G/T]	Intron NR_024434	0.024	0.032	0.62	0.042	0.333	1.50 (0.548–4.126)	0.417
17:433339921	rs34584370	[C/G]	Intron NR_024434	0.096	0.142	0.148	0.1126	0.633	1.46 (0.794–2.680)	0.207
17:43345858	rs11574833	[G/A]	Exon NR_024434	0.029	0.04	0.277	0.03	0.521	1.62 (0.638–4.155)	0.295
17:43362475	rs11574822	[C/T]	Intron MAP3K14	0.047	0.068	0.289	0.056	0.695	1.39 (0.683–2.824)	0.346
17:43453065	rs35771499 ^a	[G/C]	Intergenic (MAP3K14-ARHGAP27)	0.004	0.007	0.685	0.015	0.252	2.27 (0.252–20.409)	0.451
17:43454887	NA ^a	[G/A]	Intergenic (MAP3K14-ARHGAP27)	na	na	na	na	na	na	na
17:43757579	rs34804414	[A/C]	Intron CRHR1	0.12	0.152	0.326	0.176	0.158	1.21 (0.759–1.927)	0.448
17:43757971	rs17687796	[G/A]	Intron CRHR1	0.12	0.149	0.325	0.16	0.246	1.35(0.847–2.139)	0.231
17:43776158	rs10654221	[-/TT]	Intron CRHR1	na	na	na	na	na	na	na
17:43786683	rs76970862 ^{b,e}	[A/G]	Intron CRHR1	0.033	0.081	0.014	0.069	0.109	2.50(1.148–5.430)	0.019
17:43807649	NA ^b	[GGGGTGGC TACTCTG/-]	Intron CRHR1	na	na	na	na	na	na	na
17:43828938	rs77850169 ^b	[C/T]	Intron CRHR1	0.052	0.081	0.178	0.07	0.463	1.54 (0.787–3.023)	0.208
17:43878030	rs60645916 ^c	[G/C]	Intron CRHR1	0.069	na	na	na	na	na	na
17:43881290	rs73984616 ^c	[C/T]	Intron CRHR1	0.073	0.1	0.217	0.11	0.193	1.49 (0.853–2.601)	0.164
17:43882035	rs7222658 ^c	[A/G]	Intron CRHR1	0.073	na	na	na	na	na	na
17:43907639	rs16940662	[C/A]	Intron CRHR1	0.108	0.112	0.902	0.12	0.765	1.064 (0.638–1.774)	0.765
17:43910402	rs17689948 ^c	[G/C]	Intron CRHR1	0.073	na	na	na	na	na	na
17:43912342	rs28364032 ^b	[G/A]	UTR-CRHR1	0.073	na	na	na	na	na	na
17:43914914	rs56842912 ^c	[G/A]	Intergenic (CRHR1-SPPL2C)	0.073	0.11	0.120	0.11	0.17	1.6 (0.913–2.769)	0.100
17:43917216	rs117510714 ^d	[G/A]	Intergenic (CRHR1-SPPL2C)	0.073	0.115	0.098	0.116	0.15	1.65 (0.934–2.884)	0.079
17:43918505	rs117301738 ^d	[G/A]	Intergenic (CRHR1-SPPL2C)	0.073	0.115	0.098	0.116	0.15	1.65 (0.934–2.884)	0.079

Chr:Position	ID dbSNP	Reference/ alternative allele	Gene	Control alternative allele freq	PSP alternative allele freq	p	CBD alternative allele freq	p	OR (95% CI) for control vs. PSP + CBD	p
17:43922640	rs17763658 ^{de}	[G/A]	SPPL2C (missense)	0.073	0.115	0.098	0.116	0.15	1.65 (0.934–2.884)	0.079
17:44204606	rs78746179	[C/T]	Intron KIAA1267	0.053	0.081	0.237	0.058	0.84	1.38 (0.679–2.792)	0.357
17:44855547	rs76347593	[C/T]	Intron WNT3	0.041	0.06	0.340	0.065	0.31	1.53 (0.731–3.207)	0.28

Key: CBD, corticobasal degeneration; Chr, chromosome; CI, confidence interval; Freq, frequency; na, not available; PSP, progressive supranuclear palsy; OR, odds ratio.

^aLinkage disequilibrium blocks grouping different variants are indicated.
^bLinkage disequilibrium blocks grouping different variants are indicated.
^cLinkage disequilibrium blocks grouping different variants are indicated.
^dLinkage disequilibrium blocks grouping different variants are indicated.
^eVariants selected for the subsequent analysis in the replication sample.