Neurobiology of Aging 93 (2020) 142.e5–142.e7

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Negative results

SMPD1 variants do not have a major role in rapid eye movement sleep behavior disorder

Uladzislau Rudakou [a,b], Naomi C. Futhey [b], Lynne Krohn [a,b], Jennifer A. Ruskey [b,c], Karl Heilbron [d], Paul Cannon [d], The 23andMe Research Team[d], Armaghan Alam [b], Isabelle Arnulf [e], Michele T.M. Hu [f,g], Jacques Y. Montplaisir [h,i], Jean-François Gagnon [h,j], Alex Desautels [h,k], Yves Dauvilliers [l], Marco Toffoli [m,n], Gian Luigi Gigli [o,p], Mariarosaria Valente [m,o], Birgit Högl [q], Ambra Stefani [q], Evi Holzknecht [q], Karel Sonka [r], David Kemlink [r], Wolfang Oertel [s], Annette Janzen [s], Giuseppe Plazzi [t,u], Elena Antelmi [t,u], Michela Figorilli [v], Monica Puligheddu [v], Brit Mollenhauer [w,x], Claudia Trenkwalder [w,x], Friederike Sixel-Döring [s,w], Valérie Cochen De Cock [y,z], Christelle Charley Monaca [aa], Anna Heidbreder [bb], Luigi Ferini-Strambi [cc], Femke Dijkstra [dd,ee], Mineke Viaene [dd,ee], Beatriz Abril [ff], Bradley F. Boeve [gg], Ronald B. Postuma [b,c,h], Guy A. Rouleau [a,b,c], Ziv Gan-Or [a,b,c,*]

[a] *Department of Human Genetics, McGill University, Montréal, Quebec, Canada*
[b] *Montreal Neurological Institute, McGill University, Montréal, Quebec, Canada*
[c] *Department of Neurology and Neurosurgery, McGill University, Montréal, Quebec, Canada*
[d] *23andMe, Inc., Sunnyvale, CA, USA*
[e] *Sleep Disorders Unit, Pitié Salpêtrière Hospital, Centre de Recherche de l'Institut du Cerveau et de la Moelle Epinière, Sorbonne University, Paris, France*
[f] *Oxford Parkinson's Disease Centre (OPDC), University of Oxford, Oxford, UK*
[g] *Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, UK*
[h] *Centre d'Études Avancées en Médecine du Sommeil, Hôpital du Sacré-Cœur de Montréal, Montréal, Quebec, Canada*
[i] *Department of Psychiatry, Université de Montréal, Montréal, Quebec, Canada*
[j] *Department of Psychology, Université du Québec à Montréal, Montréal, Quebec, Canada*
[k] *Department of Neurosciences, Université de Montréal, Montréal, Quebec, Canada*
[l] *National Reference Center for Narcolepsy, Sleep Unit, Department of Neurology, Gui-de-Chauliac Hospital, CHU Montpellier, University of Montpellier, Inserm U1061, Montpellier, France*
[m] *Department of Medicine (DAME), University of Udine, Udine, Italy*
[n] *Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, London, UK*
[o] *Clinical Neurology Unit, Department of Neurosciences, University Hospital of Udine, Udine, Italy*
[p] *DMIF, University of Udine, Udine, Italy*
[q] *Sleep Disorders Clinic, Department of Neurology, Medical University of Innsbruck, Innsbruck, Austria*
[r] *Department of Neurology and Centre of Clinical Neuroscience, Charles University, First Faculty of Medicine and General University Hospital, Prague, Czech Republic*
[s] *Department of Neurology, Philipps-University Marburg, Marburg, Germany*
[t] *Department of Biomedical and Neuromotor Sciences (DIBINEM), Alma Mater Studiorum, University of Bologna, Bologna, Italy*
[u] *IRCCS, Institute of Neurological Sciences of Bologna, Bologna, Italy*
[v] *Department of Medical Sciences and Public Health, Sleep Disorder Research Center, University of Cagliari, Cagliari, Italy*
[w] *Paracelsus-Elena-Klinik, Kassel, Germany*
[x] *Department of Neurology, University Medical Centre Goettingen, Goettingen, Germany*
[y] *Sleep and Neurology Unit, Beau Soleil Clinic, Montpellier, France*
[z] *EuroMov, University of Montpellier, Montpellier, France*
[aa] *University Lille North of France, Department of Clinical Neurophysiology and Sleep Center, CHU Lille, Lille, France*
[bb] *Department of Sleep Medicine and Neuromuscular Disorders, University of Muenster, Muenster, Germany*
[cc] *Department of Neurological Sciences, Università Vita-Salute San Raffaele, Milan, Italy*
[dd] *Laboratory for Sleep Disorders, St. Dimpna Regional Hospital, Geel, Belgium*
[ee] *Department of Neurology, St. Dimpna Regional Hospital, Geel, Belgium*
[ff] *Sleep Disorder Unit, Carémeau Hospital, University Hospital of Nîmes, Nîmes, France*
[gg] *Department of Neurology, Mayo Clinic, Rochester, MN, USA*

* Corresponding author at: Montreal Neurological Institute, McGill University, 1033 Pine Avenue, West, Ludmer Pavilion, Room 312, Montreal, Quebec H3A 1A1, Canada. Tel.: +1 514 398-5845; fax: +1-514-994-8248.
E-mail address: ziv.gan-or@mcgill.ca (Z. Gan-Or).

0197-4580/$ – see front matter
https://doi.org/10.1016/j.neurobiolaging.2020.04.005

ARTICLE INFO

Article history:
Received 8 February 2020
Accepted 7 April 2020
Available online 18 April 2020

Keywords:
REM sleep behavior disorder
Sphingomyelin phosphodiesterase 1
Association study

ABSTRACT

Mutations in the sphingomyelin phosphodiesterase 1 (*SMPD1*) gene were reported to be associated with Parkinson's disease and dementia with Lewy bodies. In the current study, we aimed to evaluate the role of *SMPD1* variants in isolated rapid eye movement sleep behavior disorder (iRBD). *SMPD1* and its untranslated regions were sequenced using targeted next-generation sequencing in 959 iRBD patients and 1287 controls from European descent. Our study reports no statistically significant association of *SMPD1* variants and iRBD. It is hence unlikely that *SMPD1* plays a major role in iRBD.

1. Introduction

The sphingomyelin phosphodiesterase 1 (*SMPD1*) gene encodes the lysosomal enzyme acid sphingomyelinase, which converts sphingomyelin into ceramide. Homozygous or compound heterozygous mutations in *SMPD1* may cause Niemann-Pick disease type A (NPA) or type B, lysosomal storage disorders characterized by acid sphingomyelinase deficiency, and accumulation of sphingomyelin. In recent years, heterozygous *SMPD1* variants have been reported as risk factors for Parkinson's disease (PD) (Alcalay et al., 2019), and have been suggested to be associated with dementia with Lewy bodies. Isolated rapid eye movement (REM) sleep behavior disorder (iRBD) is a prodromal synucleinopathy, since individuals with isolated RBD are very likely to convert to PD, dementia with Lewy bodies, or multiple system atrophy (Postuma et al., 2019). In the current study, we aimed to examine whether rare and common *SMPD1* variants are associated with iRBD. A more comprehensive introduction with references can be found in the Supplementary Full Version of the Manuscript.

2. Methods

2.1. Study population

A total of 2246 subjects, composed of 959 unrelated iRBD patients and 1287 controls, were included in the study. To further investigate one variant in RBD, we examined genome-wide association study summary statistics provided by 23andMe from 1782 PD cases with probable RBD (PD + pRBD) and 131,250 age- and sex-matched controls. Additional details on the study populations can be found in the Supplementary Full Version of the Manuscript.

2.2. SMPD1 sequencing

Coding sequence and regulatory regions of *SMPD1* were targeted using molecular inversion probes, and further details on *SMPD1* sequencing can be found in the Supplementary Full Version of the Manuscript.

2.3. Quality control and statistical analysis

Quality control filtration was performed using GATK v3.8 and PLINK software v1.9. Samples with average genotyping rate of less than 90% were excluded. We excluded variants based on the following criteria: coverage quality score below 30, minimum depth of coverage which was set to 15× for common variants and 30× for rare variants, genotyping rate of less than 90%, deviation from the Hardy–Weinberg equilibrium set at $p = 0.001$, missingness difference between patients and controls set at $p = 0.05$ and adjusted by Bonferroni correction. The association between common *SMPD1* variants and iRBD was tested using a logistic regression using PLINK v1.9, adjusted for age and sex. To analyze rare variants as defined by a minor allele frequency (MAF) of less than 0.01, optimized Sequence Kernel Association Test (SKAT-O, R package) was performed. Additional information on quality control and statistical analysis is available in the Supplementary Full Version of the Manuscript.

3. Results

The average coverage of *SMPD1* was 393×, with 96% of the nucleotides covered at >20×, and 85% covered at >50×. Table 1 details the frequencies of 2 common variants, rs1050239 and rs8164, in iRBD patients and controls. Both variants were not associated with iRBD ($p = 0.48$ and $p = 0.55$, respectively; Table 1). Table 2 details rare nonsynonymous and indel variants (no splice or stop variants were identified) with MAF < 0.01 in iRBD patients and controls. The frequency of rare variants in iRBD patients was double than their frequency in controls, but the difference was not statistically significant (1.46% vs. 0.70% respectively, Fisher's exact test $p = 0.09$). There was no statistically significant burden of rare variants in *SMPD1* in iRBD (SKAT-O, $p = 0.64$). The difference in frequency of rare variants between RBD patients and control was mainly driven by the p.Ala487Val, found in 8 (0.83%) of iRBD patients and 3 (0.23%) of controls (odds ratio = 3.6, 95% confidence interval = 0.95–13.6, $p = 0.06$). However, since the control population in the current study includes controls that were used in the previous study, it is possible that by chance the frequency of this variant in our control population is low. To test this possibility, we examined data from 23andMe, comparing 1782 PD + pRBD patients

Table 1
Association of common variants in *SMPD1* with risk for iRBD

dbSNP	rs1050239	rs8164
Position	11:6415463	11:6415882
Variant	NM_000543:c.1522G>A:p.Gly508Arg	NM_000543:c.*45G>A
	n (%)	n (%)
RBD (N = 959)		
Homozygous	49 (5.11)	27 (2.82)
Heterozygous	324 (33.79)	239 (24.92)
WT	586 (61.11)	693 (72.26)
Controls (N = 1287)		
Homozygous	50 (3.89)	26 (2.02)
Heterozygous	446 (34.65)	323 (25.10)
WT	791 (61.46)	938 (72.88)
OR (95% CI)[a]	1.06 (0.90–1.24)	1.06 (0.88–1.27)
p value[a]	0.4778	0.553

Key: CI, confidence interval; dbSNP, single nucleotide polymorphism database; OR, odds ratio; RBD, rapid eye movement sleep behavior disorder; WT, wild-type.
[a] Logistic regression, additive model, adjusted for age and sex.

Table 2
Rare nonsynonymous variants and deletions detected in iRBD and controls

Position	dbSNP	Nucleotide change	Amino acid change	RBD (N = 959)	Control (N = 1287)	GnomAD MAF (non-Finnish Europeans)
11:6412055		T/C	NM_000534:p.Ile76Thr	0	1 (0.08%)	0
11:6413013		C/T	NM_000534:p.Arg240Trp	1 (0.10%)	0	0
11:6413367		G/A	NM_000534:p.Glu358Lys	1 (0.10%)	0	1.80×10^{-5}
11:6413167	rs1803161	G/A	NM_000534:p.Arg291His	2 (0.21%)	2 (0.16%)	2.2×10^{-3}
11:6413182	rs35824453	G/A	NM_000534:p.Arg296Gln	1 (0.10%)	1 (0.08%)	1.00×10^{-4}
11:6414489		C/T	NM_000534:p.Leu379Phe	0	1 (0.08%)	6.67×10^{-5}
11:6415245	rs141641266	C/T	NM_000534:p.Ala487Val	8 (0.83%)	3 (0.23%)	4.10×10^{-3}
11:6415766	rs120074118	TGCC/T	NP_000534.3:p.Arg610del	1 (0.10%)	1 (0.08%)	2.00×10^{-4}
Total				14 (1.46%)	9 (0.70%)	

Key: dbSNP, single nucleotide polymorphism database; GnomAD, Genome Aggregation Database; MAF, minor allele frequency; RBD, rapid eye movement sleep behavior disorder.

and 131,250 controls. The allele frequency of the p.Ala487Val was 0.53% in patients and 0.46% in controls, suggesting a lack of association between this variant and pRBD in PD patients (odds ratio = 1.13, 95% confidence interval = 0.68–1.88, $p = 0.64$). Full results are available in the Supplementary Full Version of the Manuscript.

4. Discussion

In the current study, we fully sequenced *SMPD1* in 959 iRBD patients and 1287 controls and identified 8 rare *SMPD1* variants (MAF < 0.01), and 2 common variants (MAF > 0.01). We found no strong evidence for an association of rare or common *SMPD1* variants with iRBD. In a previous large study of PD, the frequencies of *SMPD1* mutations in PD patients and controls of Ashkenazi Jewish origin were 1.7% and 0.4%, respectively (Alcalay et al., 2019). With our sample size, we had >80% power to detect such differences between patients and controls at $p < 0.05$. However, since our population is less homogeneous than the Ashkenazi Jewish population, we cannot rule out that rare *SMPD1* variants or variants with small effect size contribute to iRBD, and larger studies will be required in the future to conclusively determine the role of *SMPD1* in iRBD. Rare *SMPD1* variants were more frequent in cases compared to controls, yet without statistical significance. The increased frequency in patients was driven by the p.Ala487Val variant, which was previously nominally associated with PD, albeit nonsignificant after correction for multiple comparisons. To further examine whether p.Ala487Val is associated with RBD, we examined data from 23andMe comparing PD + pRBD patients and controls, which yielded statistically nonsignificant results. These results may suggest that this variant is not associated with RBD, and may further imply that the previously reported association of this specific variant with PD was also due to chance. However, we cannot rule out a minor role for this variant that requires larger cohorts and statistical power to detect. In previous studies, NPA-causing mutations, such as p.L302P and p.fsP330, have consistently been associated with PD. In the current study, we did not identify any mutation that causes NPA. Therefore, we cannot rule out that rare, thus-far undetected NPA-associated rare variants are associated with iRBD. A more elaborated discussion, including study limitations, can be found in the Supplementary Full Version of 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.2020.04.005.

References

Alcalay, R.N., Mallett, V., Vanderperre, B., Tavassoly, O., Dauvilliers, Y., Wu, R.Y.J., Ruskey, J.A., Leblond, C.S., Ambalavanan, A., Laurent, S.B., Spiegelman, D., Dionne-Laporte, A., Liong, C., Levy, O.A., Fahn, S., Waters, C., Kuo, S.H., Chung, W.K., Ford, B., Marder, K.S., Kang, U.J., Hassin-Baer, S., Greenbaum, L., Trempe, J.F., Wolf, P., Oliva, P., Zhang, X.K., Clark, L.N., Langlois, M., Dion, P.A., Fon, E.A., Dupre, N., Rouleau, G.A., Gan-Or, Z., 2019. SMPD1 mutations, activity, and alpha-synuclein accumulation in Parkinson's disease. Mov. Disord. 34, 526–535.

Postuma, R.B., Iranzo, A., Hu, M., Högl, B., Boeve, B.F., Manni, R., Oertel, W.H., Arnulf, I., Ferini-Strambi, L., Puligheddu, M., Antelmi, E., Cochen De Cock, V., Arnaldi, D., Mollenhauer, B., Videnovic, A., Sonka, K., Jung, K.Y., Kunz, D., Dauvilliers, Y., Provini, F., Lewis, S.J., Buskova, J., Pavlova, M., Heidbreder, A., Montplaisir, J.Y., Santamaria, J., Barber, T.R., Stefani, A., St Louis, E.K., Terzaghi, M., Janzen, A., Leu-Semenescu, S., Plazzi, G., Nobili, F., Sixel-Doering, F., Dusek, P., Bes, F., Cortelli, P., Ehgoetz Martens, K., Gagnon, J.F., Gaig, C., Zucconi, M., Trenkwalder, C., Gan-Or, Z., Lo, C., Rolinski, M., Mahlknecht, P., Holzknecht, E., Boeve, A.R., Teigen, L.N., Toscano, G., Mayer, G., Morbelli, S., Dawson, B., Pelletier, A., 2019. Risk and predictors of dementia and parkinsonism in idiopathic REM sleep behaviour disorder: a multicentre study. Brain 142, 744–759.