HHS Public Access

Author manuscript

Parkinsonism Relat Disord. Author manuscript; available in PMC 2016 January 23.

Published in final edited form as:

Parkinsonism Relat Disord. 2014 June ; 20(6): 584. doi:10.1016/j.parkreldis.2014.02.021.

Does α-Synuclein Have A Dual and Opposing Effect in Preclinical versus Clinical Parkinson's Disease?

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Abstract

α-Synuclein gene (*SNCA*) multiplications cause familial parkinsonism and allele-length polymorphisms within the *SNCA* dinucleotide repeat REP1 increase the risk for developing Parkinson's disease (PD). Since *SNCA* multiplications increase *SNCA* expression, and REP1-genotypes that increase the risk of developing PD show increased *SNCA* expression in cell-culture systems, animal models, and human blood and brain, PD therapies seek to reduce *SNCA* expression. We conducted an observational study of 1,098 PD cases to test the hypothesis that REP1 genotypes correlated with reduced *SNCA* expression are associated with better motor and cognitive outcomes. We evaluated the association of REP1 genotypes with survival free of Hoehn and Yahr stages 4 or 5 (motor outcome) and of Modified Telephone Interview for Cognitive Status score ≤27 or Alzheimer's Disease-8 score ≥2 (cognitive outcome). Median disease duration at baseline was 3.3 years and median lag time from baseline to follow-up was 7.8 years. Paradoxically, REP1 genotypes associated with increased risk of developing PD and increased *SNCA* expression were associated with better motor (HR=0.87, p=0.046 covariate-adjusted age-scale analysis; HR=0.85, p=0.020, covariate-adjusted time-scale analysis) and cognitive outcomes (HR=0.90, p=0.12, covariate-adjusted age-scale analysis; HR=0.85, p=0.023, covariate-adjusted

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time-scale analysis). Our findings raise the possibility that *SNCA* has a dual, opposing, and time-dependent role. This may have implications for the development of therapies that target *SNCA* expression.

Keywords

Parkinson's disease; α-synuclein gene; outcomes

INTRODUCTION

α-Synuclein gene (*SNCA*) multiplications cause familial PD via an overexpression mechanism [1]. PD susceptibility is influenced by allele-length polymorphisms in the mixed dinucleotide repeat REP1 (D4S3481, ~10 kb upstream of the *SNCA* transcription start site) [2], which are correlated with altered *SNCA* expression in cell cultures [3], transgenic mouse brain [4], and human blood and brain [5–6]. Specifically, longer REP1 allele lengths (263 bp) that correlate with increased *SNCA* expression are associated with increased risk of developing PD and shorter REP1 allele lengths (259 bp) that correlate with reduced *SNCA* expression are associated with reduced risk of developing PD [2]. These genetic findings, together with α-synuclein immunostaining of Lewy bodies [7], provide proof of principle for therapies aiming to reduce *SNCA* expression in PD [8–9].

Recently, Ritz et al. [10] reported the association of *SNCA* REP1 263 bp alleles (correlated with increased expression) with more rapid motor symptoms progression in PD as assessed by the rate of decline of Unified Parkinson's Disease Rating Scale (UPDRS) motor scores over a 5-year interval. They interpreted their findings to support therapies targeting reduction of *SNCA* expression in PD. Here we present a study of the association of twenty *SNCA* variants (REP1 and 19 additional haplotype-tagging SNPs) and survival free of motor and cognitive outcomes, for 1,098 PD subjects with up to 13 years of follow-up. Since increased *SNCA* expression is associated with increased risk of developing PD, we hypothesized that REP1 genotypes correlated with reduced *SNCA* expression (one or two 259 bp alleles) would be associated with greater survival free of developing motor and cognitive outcomes in PD. Surprisingly, the findings of our studies are opposite to this hypothesis and contrast with those of Ritz et al. and suggest a possible time-dependent, dual and opposing effect of *SNCA* in PD.

MATERIALS AND METHODS

Subjects

Clinical information and biological samples were collected with written informed consent following a protocol approved by the Mayo Clinic IRB (Rochester, MN). Study subjects were 1,098 PD cases from the Molecular Epidemiology of Parkinson's Disease study ("MEPD study", NIH 2R01ES10751) referred sequentially to the Department of Neurology of the Mayo Clinic in Rochester, MN, from June 1, 1996 through June 30, 2007. They resided in Minnesota or a neighboring state. All PD cases were examined in a standardized fashion by neurologists specializing in Movement Disorders, and employing a

comprehensive protocol for clinical assessment. Cases fulfilled criteria for clinically definite or probable PD [11].

Molecular analyses

Blood was collected and genomic DNA was obtained. Allele length of *SNCA* REP1 was assessed using an Applied Biosystems sequencing platform (Genotyping Shared Resources, Mayo Clinic, Rochester, MN) and 19 haplotype-tagging *SNCA* SNPs were assessed using an Illumina GoldenGate genotyping platform [12].

Outcome measurements

Motor and cognitive outcome data for 1,098 PD cases were collected by telephone interview questionnaires directly with the cases or via proxy when incapacitated or deceased. The direct interview questionnaire included the Telephone Interview of Cognitive Status-Modified (TICS-M) [13] and questions regarding motor milestones such as inability to stand or walk unassisted (and dates). The proxy questionnaires collected the same information, with the exception of screening for dementia using the Alzheimer's Disease Dementia Screening Interview (AD-8) [14] because AD-8 is a brief informant-based measure that reliably differentiates between non-demented and demented individuals and is sensitive to the earliest signs of cognitive change as reported by a proxy informant.

The motor outcome was defined as Hoehn and Yahr (H&Y) stage 4 or 5 and assessed at baseline via clinical assessment and imputed at follow-up via telephone interview. The question asked at the telephone interview was: "Are you able to stand or walk without someone else helping you, or without a cane or walker?" A "no" response corresponded to H&Y stages 4 or 5. A "no" response was followed by the question: "At what age were you no longer able to stand or walk without assistance?" Both questions were appropriately reworded for proxy interviews.

Cognitive outcome was assessed at baseline using the Mini Mental State Examination (MMSE). The cognitive outcome was defined as MMSE <26 [15]. Cognitive outcome at follow-up was assessed via telephone interview using the TICS-M (direct interviews) or the AD-8 (proxy interviews). Cognitive outcome was defined as a TICS-M score ≤27 or AD-8 score ≥2. The outcome assessments were identical to those that we reported for a genome-wide association (GWAS) study of motor and cognitive outcomes in PD [16]. That study did not include *SNCA*-REP1 genotypes and the 19 *SNCA* haplotype-tagging SNPs that are the focus of this study, and that study included only a subset (n=443) of the cases included in this study (n=1,098).

Statistical analyses

Statistical analyses evaluated the association of each genetic variant with motor and cognitive impairment, with the primary outcome being time-to-event (motor or cognitive outcomes). Cox proportional hazards regression models were used to assess the association of outcomes with genotypes. For cases with no evidence of motor or cognitive outcomes, time-to-censoring was defined as the time between the baseline clinical assessment and the telephone interview, or time between baseline and death if the individual was deceased. For

cases with evidence of motor or cognitive outcomes, time-to-event was defined based on the age of onset of motor or cognitive outcomes as determined by direct or proxy telephone interviews. Subjects with H&Y stage 4/5 or with MMSE <26 at baseline, were excluded from the respective motor or cognitive outcomes survival analyses. To limit survival bias, age at enrollment was considered as time 0 (or baseline). Analyses were performed both using the age-scale and the time-on-study scale. While the time-on-study scale is often used in analyses of disease progression, analysis on the age scale is more appropriate when studying outcomes associated with age in an aging population [18]. Log-rank tests were used to determine significance and associations between outcomes and genetic variables using hazard ratios (HR) with 95% confidence intervals. Kaplan-Meier plots were used to visualize the overall survival function, and stratified by genetic variables.

Prior to testing the effects of SNCA variants on motor and cognitive impairment outcomes, analyses were performed to identify relevant covariates associated with the outcomes. Relevant covariates were then included in the Cox proportional hazards models that were used to assess the association of outcomes with genotypes. The age-scale analysis of the motor impairment outcome included sex, disease duration at baseline, and L-DOPA treatment at baseline as covariates, while the cognitive impairment analysis included sex, disease duration at baseline, and education as covariates. The age-scale analyses of motor and cognitive outcomes were also performed without covariate adjustment. Similarly, the analyses on the time-on-study scale were performed without and with covariate adjustment (same covariates as above with the addition of age at enrollment as a covariate in analyses of both motor and cognitive outcomes).

The primary analysis included the association of outcomes with *SNCA* REP1 genotypes using the REP1 score as described previously [17]. The REP1 score ranged from 0 (lowest PD risk, lowest *SNCA* expression) to 4 (highest PD risk, highest *SNCA* expression). Secondary analyses investigated the association of motor and cognitive outcomes with 19 haplotype-tagging *SNCA* SNPs defined by the minor allele count (0, 1, or 2 copies of the minor frequency allele); and with REP1 genotypes re-defined by minor allele counts (0, 1, or 2 copies of the 259 bp allele; or 0, 1, or 2 copies of the 263 bp allele). As *SNCA* REP1 was the genetic polymorphism of interest, primary analyses were not corrected for multiple comparisons. The Bonferroni correction for multiple comparisons was applied to the secondary analyses (20 tests for each outcome). Results with uncorrected p-values < 0.05 in the primary analyses or Bonferroni-corrected p-values < 0.05 in the secondary analyses were considered significant.

The statistical packages SAS® (version 9.2; SAS Institute Inc., Cary, NC) and R (version 2.14; R Development Core Team (2011). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/) were used for all analyses.

RESULTS

Demographic and clinical characteristics

1,098 MEPD cases were included in this study (Supplemental Table 1). 85 cases were lost to follow-up, and 91 PD cases (or proxies) refused follow-up interviews. Thus, the overall participation rate among those contacted was 91.0%. At follow-up, 467 direct interviews, 180 proxy interviews for incapacitated subjects, and 275 proxy interviews for deceased subjects were performed. From the 922 participating cases 604 were men (65.5%) and 318 women (34.5%) primarily of Caucasian race and European ancestry. Median disease duration at baseline was 3.3 years. 652 of the 922 participating cases (70.7%) had been treated with levodopa at baseline. 62 cases (6.7%) that had reached H&Y stages 4/5 at baseline were removed from the motor outcomes analyses. 59 cases (6.4%) that had reached MMSE <26 at baseline were removed from the cognitive analyses.

The median lag time from baseline to follow-up was 7.8 years (range 3.3–13 years). 44 cases with a TICS-M score ≤27 were interviewed directly and repeated interviews (proxy for incapacitated subjects) were conducted in order to obtain valid dates and other information for the survival analyses. Information regarding levodopa therapy at follow-up or cumulative dose exposures was not available.

Molecular analysis

Twenty *SNCA* variants were genotyped and their positions are shown in a Linkage Disequilibrium (LD) map for *SNCA* (Supplemental Figure 1).

REP1 and motor outcomes

Our initial hypothesis was that PD cases with lower REP1 scores, associated with reduced PD risk and correlated with reduced *SNCA* expression, would have reduced risk of developing motor and/or cognitive impairment and thus longer survival free of these outcomes. However, we observed the opposite association: PD cases with higher REP1 scores had reduced risk of developing motor impairment and longer survival free of the outcome. This result was statistically significant both in the unadjusted and the covariate-adjusted analyses on the time-on-study scale as well as the unadjusted and covariate-adjusted analyses performed on the age-scale (adjusted analysis on age-scale: HR = 0.87, 95% CI 0.75–1.00, p = 0.046; see Supplemental Table 2). Under the covariate-adjusted age-scale model, having a lower REP1 score conferred a nearly two-fold greater risk for developing the motor outcome than a higher REP1 score (HR=1.78, 95%CI 1.01–3.15, REP1 score of 0 vs. REP1 score of 4). Figure 1 shows the Kaplan-Meier plot for survival free of developing the motor outcome by the REP1 score. Genotype and allele frequencies are shown in Table 1.

REP1 and cognitive outcomes

Consistent with our findings for motor outcomes, we observed an opposite-than-expected association for cognitive outcomes. PD cases with higher REP1 scores had reduced risk of developing cognitive impairment and longer survival free of the outcomes. While this result was statistically significant in the unadjusted and covariate-adjusted analysis on the time

scale (adjusted analysis on time-scale: HR = 0.85, 95% CI 0.75–0.98, p = 0.023), it just failed to achieve significance (p=0.058) in the unadjusted analysis on the age-scale, and was not significant in the covariate-adjusted analysis on the age-scale (p=0.12). Under the covariate-adjusted age-scale model, having a low REP1 score conferred a 1.5 times greater risk for developing the cognitive outcome than having a high REP1 score (HR=1.53, 95% CI 0.90–2.60, REP1 score of 0 vs. REP1 score of 4). Results of all analyses are shown in Supplemental Table 3. Figure 2 shows the Kaplan-Meier plots for survival free of the cognitive outcome by REP1 score. Genotype and allele frequencies are shown in Table 2.

SNPs and motor and cognitive outcomes

The association results for all genotyped *SNCA* variants (19 SNPs, REP1) and developing the motor and cognitive outcomes are included in Supplemental Tables 2 and 3. None of the SNPs were significantly associated with developing motor or cognitive impairment after Bonferroni correction for multiple testing, including those tagging the 3′ LD block.

DISCUSSION

In contrast to our original hypothesis our results reveal an unexpected association of the *SNCA* REP1 genotypes correlated with reduced *SNCA* expression with the development of worse motor and cognitive outcomes in a large and well-characterized cohort of PD cases. Our results are inconsistent with findings from *SNCA* multiplication mutations in familial parkinsonism, where increased *SNCA*-genomic dosage and *SNCA* expression were associated with earlier age at onset, faster progression and increased motor and cognitive severity [1]. However, our results are consistent with clinical and genetic information that two of the authors (KM, BAC) newly obtained from a previously reported Greek family with PD [19]. In affected kindred members, the *SNCA* c.157G>A (p.A53T) mutation is in phase with the REP1 259-bp allele (Supplemental Figure 2). All affected individuals with the 259-bp REP1 allele had a severe phenotype with poor motor and cognitive outcomes (median duration to H&Y stages 4/5 was 7 years and to dementia was 7 years), while a recombinant lacking the 259-bp REP1 allele remains asymptomatic past the mean age of onset for their generation. This is consistent with the hypothesis that the co-localization of the causal gene mutation with the 259-bp REP1 allele is associated with variable expressivity (worse outcomes) in this family.

Our results are also inconsistent with findings reported by Ritz et al [10], where REP1 alleles associated with increased PD risk and correlated with increased *SNCA* expression were associated with the development of worse motor outcomes. There are several possible explanations for these conflicting results. First, the difference may be due to sample size. Our sample at study enrollment was 3-fold larger than that of Ritz et al. (1,098 vs. 363 PD cases), and at follow-up was nearly 4-fold larger (854 vs. 233 PD cases). Small and underpowered samples have greater false-positive rates and may provide erroneous or exaggerated estimates of the direction or size of effect. Second, the population characteristics were different. Our study population was racially and ethnically homogeneous with subjects of Caucasian and European descent, whereas in the Ritz study ~19% of the subjects were non-Caucasian. The frequencies and effects of gene variants vary

by race and ethnicity, and population stratification can further erode statistical power or bias towards false-positive associations. Third, we defined motor outcome differently. Our outcome was reaching H&Y stages 4/5; inability to stand or walk unassisted represents a major milestone in PD progression and is therefore likely to be recalled reliably by patients or their proxies. Ritz et al. used the rate of decline in motor severity measured by the UPDRS part III scale. The UPDRS is subject to treatment effects and inter-rater variability, and change in the UPDRS score by five points may not result in a meaningful change in H&Y stage. Our findings were internally consistent for the development of both motor and cognitive outcomes, whereas the Ritz study did not assess cognitive measures. Fourth, our follow-up duration was substantially longer, nearly fifteen years vs. five years of Ritz et al. It is conceivable that we are observing differential effects of REP1-allele genotypes at different disease stages.

While a cross-sectional study of *SNCA* polymorphisms and the development of motor outcomes in a cohort of multiplex families with PD has been reported, it was not representative of sporadic PD [20]. Association studies of *SNCA* polymorphisms and cognitive outcomes in sporadic PD were small, considered only a few variants, and were mostly cross-sectional [21–23].

Our genetic association study has several strengths: First, we used an observational study design to evaluate the effects of *SNCA* genotypes on the development of motor and cognitive outcomes in PD. While there have been experimental studies of therapies targeting *SNCA* expression in model systems [8–9], observational studies of *SNCA* effects on the development of motor and cognitive outcomes to assess the long term benefits of therapeutic interventions are lacking. Second, all MEPD study cases were recruited using strict enrollment and diagnostic criteria, resided in a defined geographic region, and examined at baseline by Movement Disorders specialists using a standardized and comprehensive clinical assessment protocol. Third, our follow-up telephone interview assessments included validated measures allowing us to determine outcomes directly or by proxy for 84% of the subjects. Fourth, follow-up interval was long (up to 13 years) and the study had sufficient power to detect clinically meaningful effects. Fifth, genotypes were determined for REP1 but also for 19 *SNCA* SNPs.

Our study also has several weaknesses. First, a sampling bias cannot be excluded as our PD cohort was referral-based. Attempts to limit sampling bias included recruiting cases prospectively from a defined geographic region and by not excluding or enriching for familial PD cases. The frequency of known PARK mutations was very low [24–25]. Second, to limit survival bias we evaluated outcomes in PD cases with variable disease duration at baseline and included disease duration at baseline as an adjustment variable in the statistical models. Third, the measurements at baseline and follow-up employed different methods and the validity of self or proxy reported assessments and imputed H&Y stages are unclear. Fourth, treatment effects cannot be excluded since our PD cohort was not randomized to treatment. L-DOPA therapy at baseline was included as an adjustment variable in some of the models, but cumulative dose exposures to dopaminergic therapy could not be ascertained. Fifth, statistical power may be greater for repeated quantitative measures than for singly performed dichotomous measures and survival analyses.

Both a neurotoxic and a neuroprotective role for *SNCA* expression have been proposed in cell culture and animal models [26]. In addition, both up-regulation and down-regulation of *SNCA* expression in PD and control brains has been reported [6, 27–29]. It is conceivable that *SNCA* expression variability may reflect a dynamic state of *SNCA* regulation over the disease course. *SNCA* REP1 genotypes associated with reduced expression may function in a neuroprotective manner reducing the risk to develop PD, whereas well after PD symptom-onset these genotypes may exacerbate disease progression. One speculative mechanism is that after disease onset, as α-synuclein and its proteolytic fragments are sequestered in aggregates (e.g., Lewy Bodies), relatively reduced expression fails to support the production of sufficient pools of normal α-synuclein, which in turn contributes to further disease progression. Individuals with short REP1 alleles may be more at-risk for this condition following disease onset. A dual and opposing effect of gene/protein function in neurological disease is not limited to *SNCA*/α-synuclein. β-Amyloid, which plays a critical role in the pathogenesis of Alzheimer's disease, has a beneficial effect in animal models of multiple sclerosis [30, 31]. The apolipoprotein E gene ε2 variant (*APOE* ε2) is associated with a reduced risk for Alzheimer's disease, but with an increased amyloid neuropathology burden in Alzheimer's disease patients older than 90 years; and while *APOE* ε2 reduces the risk for Alzheimer's disease, it increases the risk for cerebral amyloid angiopathy and associated hemorrhages (which are prevalent in Alzheimer's disease patients) [32]. Further, *APOE*ε4 alleles have differential effects on amyloid load and glucose metabolism in the frontal and occipital cortices of Alzheimer's disease patients [33].

Taken together, these observations point to a paradigm for neurodegeneration in which a critical gene's function depends on its spatial and temporal context, potentially resulting in dual and opposing effects. Our findings suggest that therapies aiming at SNCA reduction may worsen later outcomes. These effects may not be detected by Phase 1 clinical trials that select small samples of early PD cases and employ short follow-up periods. Observational studies using large and representative PD cohorts with long follow-up periods may evaluate this critical issue.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This paper was made possible by the grant 2R01ES10751 from the National Institutes of Health, and by a grant from Alnylam Pharmaceuticals, Inc. and Medtronic, Inc. (to Demetrius M. Maraganore, MD). Matthew J. Farrer, PhD acknowledges support from the Canada Excellence Research Chairs Program and Leading Edge Endowment Funds provided by the Province of British Columbia, Life Labs, and Genome BC. Work on Family H was supported in part by a grant from the National Parkinson Foundation (to Bruce A. Chase, PhD and Katerina Markopoulou MD, PhD), a seed grant from the University of Nebraska Medical Center (to Katerina Markopoulou MD, PhD), and from the National Institutes of Health grant R15NS043162-01A2 (to Bruce A. Chase, PhD).

Abbreviations

AD-8 Alzheimer's Disease-8

bp	base pair
H&Y	Hoehn and Yahr
HR	hazard ratio
LD	linkage disequilibrium
MEPD	Molecular Epidemiology of Parkinson's Disease
MMSE	Mini Mental State Examination
SNCA	α-synuclein gene
SNP	single-nucleotide polymorphism
TICS-M	Modified Telephone Interview for Cognitive Status
UPDRS	Unified Parkinson's Disease Rating Scale

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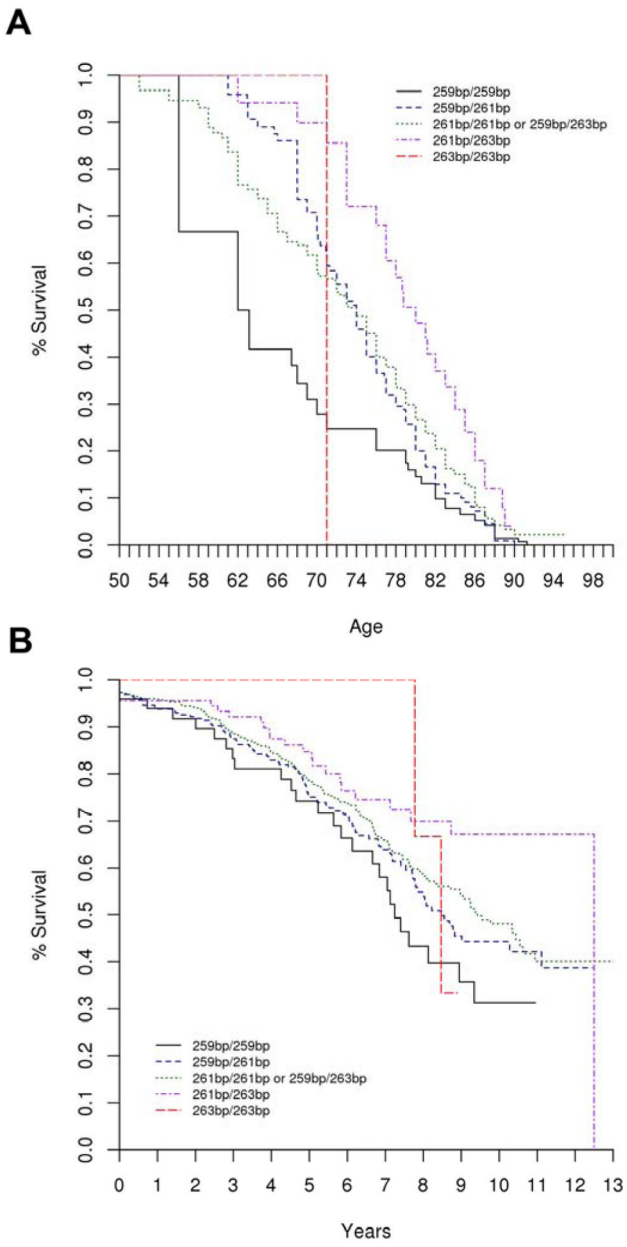

Figure 1.

Kaplan Meier plot for *SNCA* REP1 and survival free of motor impairment (Hoehn and Yahr stages 4 or 5). The genotype 259bp/259bp corresponds to REP1 score = 0, and 259bp/261bp to REP1 score = 1, and 261bp/261bp or 259bp/263bp to REP1 score = 2, and 261bp/263bp to REP1 score = 3, and 263bp/263bp to REP1 score = 4. Figure 1a corresponds to the covariate unadjusted, age-scale analysis (HR = 0.87, 95% CI 0.75–0.99, p = 0.042); and Figure 1b corresponds to the covariate unadjusted, time-on-study analysis (HR = 0.81, 95% CI 0.70–0.93, p = 0.0035).

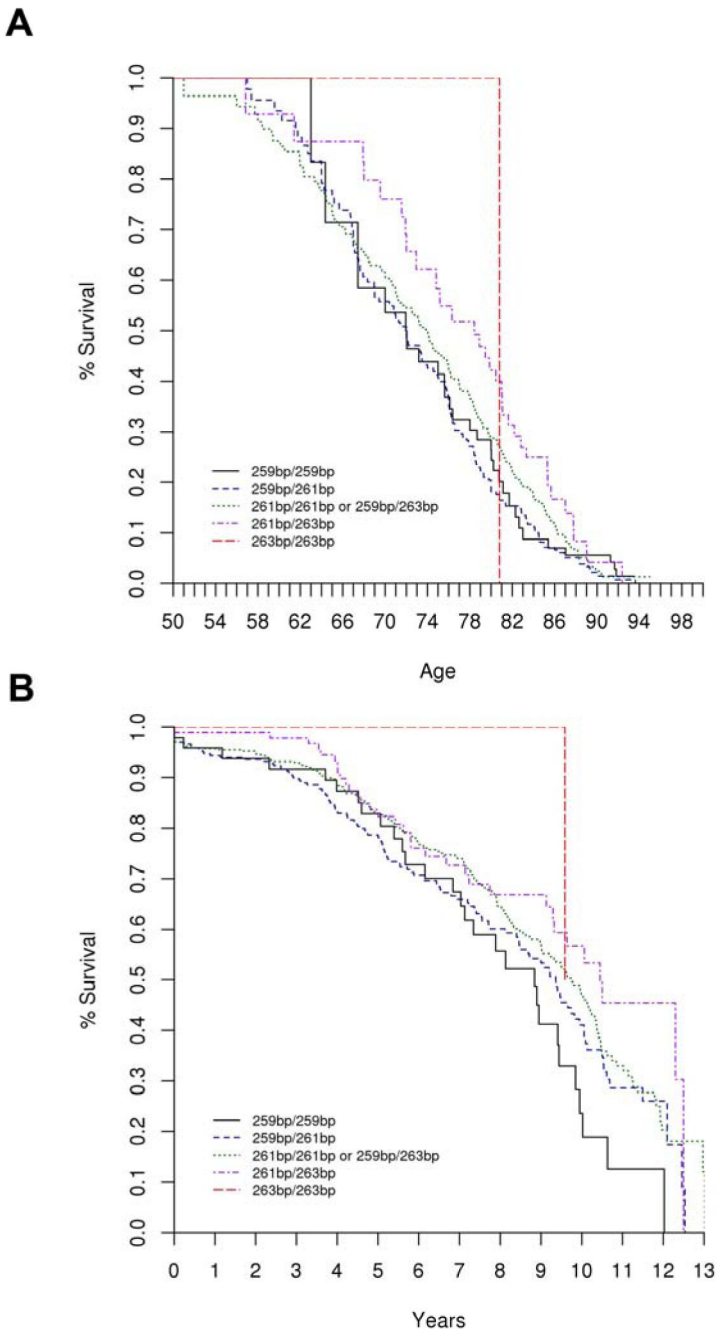

Figure 2.

Kaplan Meier plot for *SNCA* REP1 and survival free of cognitive impairment (TICS-M score ≤27 or AD-8 score ≥2). The genotype 259bp/259bp corresponds to REP1 score = 0, and 259bp/261bp to REP1 score = 1, and 261bp/261bp or 259bp/263bp to REP1 score = 2, and 261bp/263bp to REP1 score = 3, and 263bp/263bp to REP1 score = 4. Figure 2a corresponds to the covariate unadjusted, age-scale analysis (HR = 0.88, 95% CI 0.78–1.00, p

= 0.058); and Figure 2b corresponds to the covariate unadjusted, time-on-study analysis (HR = 0.81, 95% CI 0.71–0.92, p = 0.0017).

Table 1

REP1 genotype frequencies for MEPD Motor outcomes (N=854)

REP1 genotypes	**Genotype Frequency n (%)**
257/261	2 (0.2%)
257/263	1 (0.1%)
259/259	49 (5.7%)
259/261	257 (30.1%)
259/263	25 (2.9%)
259/265	1 (0.1%)
261/261	422 (49.4%)
261/263	90 (10.5%)
261/265	1 (0.1%)
263/263	6 (0.7%)
REP1 score	**Genotype Frequency n (%)**
0	49 (5.8%)
1	257 (30.3%)
2	447 (52.7%)
3	90 (10.6%)
4	6 (0.7%)
REP1 259 bp	**Genotype Frequency n (%)**
0	522 (61.1%)
1	283 (33.1%)
2	49 (5.7%)
REP1 263 bp	**Genotype Frequency n (%)**
0	732 (85.7%)
1	116 (13.6%)
2	6 (0.7%)
REP1 261 bp	**Genotype Frequency n (%)**
0	82 (9.6%)
1	350 (41.0%)
2	422 (49.4%)

Table 2

REP1 genotype frequencies in cognitive outcomes (N=858)

REP1 genotypes	**Genotype Frequencies n (%)**
257/261	2 (0.2%)
257/263	1 (0.1%)
259/259	48 (5.6%)
259/261	265 (30.9%)
259/263	25 (2.9%)
259/265	1 (0.1%)
261/261	416 (48.5%)
261/263	94 (11.0%)
261/265	1 (0.1%)
263/263	5 (0.6%)
REP1 score	**Genotype Frequency n (%)**
0	48 (5.6%)
1	265 (31.1%)
2	441 (51.7%)
3	94 (11.0%)
4	5 (0.6%)
REP1 259 bp	**Genotype Frequency n (%)**
0	519 (60.5%)
1	291 (33.9%)
2	48 (5.6%)
REP1 263 bp	**Genotype Frequency n (%)**
0	733 (85.4%)
1	120 (14.0%)
2	5 (0.6%)
REP1 261 bp	**Genotype Frequency n (%)**
0	80 (9.3%)
1	362 (42.2%)
2	416 (48.5%)