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Lack of association between the *CCR5*-delta32 polymorphism and neurodegenerative disorders

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Abstract

Objective: Recent studies have suggested that diminished Ccr5 functioning have an effect on synaptic plasticity and hippocampal memory in mouse models. *CCR5*-delta32, a 32-bp frameshift deletion in human *CCR5* encoding a nonfunctional receptor, has been reported to have a protective effect against HIV infection but its role as a modifier of neurodegenerative disease has been minimally explored. We investigated whether the *CCR5*-delta32 polymorphism could have an effect in the context of human neurodegenerative diseases.

Methods: We examined the frequency of the *CCR5*-delta32 polymorphism in a large and well-characterized cohort including 1,425 patients with neurodegenerative dementias and 2,032 controls.

Results: We did not observe a significant association between the *CCR5*-delta32 polymorphism and any of the neurodegenerative diseases screened in this study. However, we observed an earlier age of onset among neurodegenerative disease patients carrying the *CCR5*-delta32 allele.

Conclusions: While our findings were inconclusive, the earlier age of onset observed among neurodegenerative disease patients carrying the *CCR5*-delta32 allele suggests that the deletion may have a detrimental effect in the context of neurodegeneration.

Introduction

The *CCR5* gene encodes the chemokine receptor type 5, a G-protein-coupled receptor expressed in T-cells, dendritic cells, and microglia, that is involved in recruiting leukocytes to inflammatory sites. CCR5 is also a co-receptor for the human immunodeficiency virus type 1 (HIV-1) viral entry.[1] A 32-bp frameshift deletion resulting in a nonfunctional receptor (CCR5-delta32), predominantly seen in individuals of European descent (12.3% allele

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frequency in the 1,000 Genomes Project)[2], has been extensively studied for its role in HIV transmission and inflammation[3,4] and is known to confer a protective effect against HIV transmission.[5] Recently, decreased Ccr5 function has been shown to affect learning and memory in mice.[6,7] Several groups have also examined the role of *CCR5*-delta32 in Alzheimer's disease (AD), reporting no significant findings.[8-12] However, a large cohort including non-Alzheimer's neurodegenerative dementias has not been studied. The aim of this study was to assess the frequency of the *CCR5*-delta32 polymorphism in a cohort of 1,425 patients with neurodegenerative diseases and 2,032 healthy control subjects.

Methods

Participants.

We included 1,425 patients clinically diagnosed with neurodegenerative diseases – AD, amyotrophic lateral sclerosis (ALS), corticobasal syndrome (CBS), frontotemporal dementia (FTD) spectrum disorders (including behavioral variant FTD, non-fluent and semantic variants of primary progressive aphasia), mild cognitive impairment (MCI), and progressive supranuclear palsy syndrome (PSP-S) – recruited at the Memory and Aging Center at the University of California, San Francisco (UCSF-MAC). Patients consisted of individuals of 86.2% European ancestry, 1.4% African ancestry, 10% Asian ancestry, and 2.3% mixed ancestry. The majority of our control group consisted of aged samples obtained from the NIMH Human Genetics Initiative (n=1,622). The remaining control samples (n=410) were recruited at the UCSF-MAC and inclusion criteria included: availability of a reliable study partner with frequent contact, Clinical Dementia Rating (CDR) score of zero, no subject or informant report of significant cognitive decline during the previous year, no evidence from the screening visit suggesting a neurodegenerative disorder (per the team's clinical judgment), and MMSE score >25. Controls consisted of individuals aged 65 or older, European (74.3%), African (21.1%), Asian (3.9%), and mixed (0.7%) ancestry. All individuals (or their surrogates) provided authorization for genetic testing research.

Genetic analysis.

CCR5-delta32 (rs333) genotypes were obtained using a custom TaqMan® SNP assay (#AHUAPPK) from ThermoFisher on a LightCycler® 480 System, following the standard end-point genotyping protocol provided by the manufacturer. Statistical analysis was performed in R (version 3.1.3, www.r-project.org). Statistical significance was established at $P < 0.05$ for primary analysis of all neurodegenerative cases versus controls.

Results

We identified 231 *CCR5*-delta32 heterozygous carriers and 23 individuals homozygous for the deletion among 1,425 neurodegenerative patients. 336 *CCR5*-delta32 heterozygous carriers and 25 individuals homozygous for the deletion were observed among 2,032 control subjects (Table 1).

Combined analysis on the 1,425 patients placed the estimated odds ratio at 1.03 (C.I.: 0.87—1.21, Fisher's exact test, $P = 0.7715$) for overall neurodegenerative diseases versus controls (Figure 1). Analyses of individual disease groups produced odds ratios ranging from 0.96 (in

the AD group) to 1.30 (in the CBS group), none reaching statistical significance. Similar results were obtained after restricting the analysis to individuals of European descent (Supplementary Table 1, Supplementary Figure 1). Similarly, we observed no significant difference between neurodegenerative cases and controls when comparing genotype groupings (Pearson's chi-squared test, $P = 0.6242$).

A recent study has shown that murine Ccr5 is important in suppressing cortical plasticity and is involved in hippocampal learning and memory via the MAPK/CREB pathway.[6] Therefore, we also examined the effect of *CCR5*-delta32 on neurodegenerative disease age of onset, as a potential endophenotype reflecting affected neural plasticity among *CCR5*-delta32 carriers. Across all disease cohorts, we found that *CCR5*-delta32 polymorphism had a significant association with an earlier age of onset both in the overall cohort (Wilcoxon rank sum test with continuity correction, $P = 0.0164$; Welch two sample t-test, $P = 0.03166$), and in the individuals only of European descent ($P = 0.02804$; $P = 0.05226$) (Figure 2 and Supplementary Figure 2).

Discussion

In this study, we investigated the effect of the *CCR5*-delta32 polymorphism in a large and diverse neurodegenerative disease patient cohort. While we did not find an association between the *CCR5*-delta32 polymorphism and any of the neurodegenerative diseases we screened, we observed an earlier age of onset across all neurodegenerative patients carrying the *CCR5*-delta32 polymorphism. Since the number of individuals with age of onset information available was limited, we did not have statistical power to examine age of onset for each individual disorder.

In accordance to other *CCR5*-delta32 studies in Italian, Spanish and Iranian populations, [8-12] our data also showed no statistically significant differences between AD and control groups. While we found no association with the other neurodegenerative diseases investigated herein, it should be noted that statistical power was limited for some of our series (106 PSP-S 106, 79 CBS and 25 ALS) due to small sample size, and therefore larger cohorts are necessary to assert the effect of *CCR5*-delta32 in these diseases. In addition, population stratification is a potential confounder in association studies, where ethnic variation or other confounding factors can lead to significant population differences in marker allele frequencies. Indeed, allele frequencies for *CCR5*-delta32 does vary across populations (with less than 1% in Asians and Africans and 3.3% in admixed American) with highest frequency among European populations (12.3%).[2] In order to address for population stratification in our study we have also examined solely the European individuals, where similar results were obtained. Ultimately, even though we found no association with *CCR5*-delta32, it doesn't necessary imply lack of causality as there might be other(s) variant(s) in *CCR5* that could influence the risk of developing neurodegeneration.

Zhou et al. have shown that murine Ccr5 is important in suppressing cortical plasticity and is involved with hippocampal learning and memory via the MAPK/CREB pathway.[6] Based on these findings, it could be expected that the human nonfunctional CCR5 receptor, caused by the *CCR5*-delta32 polymorphism, would have a protective effect against neurodegeneration,

and therefore result in a delayed onset of neurodegenerative phenotype. However, in our cohort, we observed an earlier age of onset among patients with the *CCR5*-delta32 polymorphism, suggesting the deletion may actually have a detrimental effect in the context of neurodegeneration. This is consistent with older murine studies, where Ccr5 deficiency lead to astrocytes activation causing Abeta deposit, and memory function impairment.[7] Furthermore, a recent study has shown that homozygous delta-32 deletion seems to be deleterious in humans, as it is associated with reduced life expectancy in over 400,000 British individuals.[13]

Since some of our data seems to suggest a detrimental effect of CCR5 deficiency on neurodegeneration, further studies are warranted to investigate the exact role of the *CCR5*-delta32 polymorphism in neurodegeneration and elucidate whether it may modify age of onset or disease course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Cohort	#Individuals	#WT Alleles	#D32 Alleles	Odds Ratio	P−value
AD	517	939	95	0.96	0.8117
FTD	419	756	82	1.03	0.7963
MCI	279	505	53	1	1
PSP−S	106	189	23	1.16	0.4741
CBS	79	139	19	1.3	0.272
ALS	25	45	5	1.06	0.8091
Control	2032	3678	386		
Overall	**1425**	**2573**	**277**	**1.03**	**0.7715**

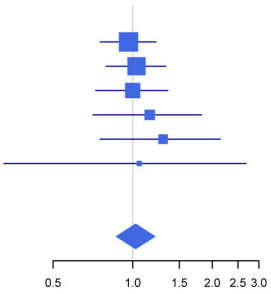

Figure 1: *CCR5*-delta32 allelic frequencies and associated odds ratios for all disease cohorts.

Total number of individuals and *CCR5*-delta32 carriers for each disease cohort and control group is shown in the table (*left*), with odds ratios and *P*-values represented in the forest plot (*right*). AD: Alzheimer's disease, FTD: frontotemporal dementia, MCI: mild cognitive impairment, PSP-S: progressive supranuclear palsy syndrome, CBS: corticobasal syndrome, ALS: amyotrophic lateral sclerosis. Overall refers to the combined group of neurodegenerative disease samples. In the forest plot, squares are drawn proportional to n in each series, and lines represent 95% confidence intervals.

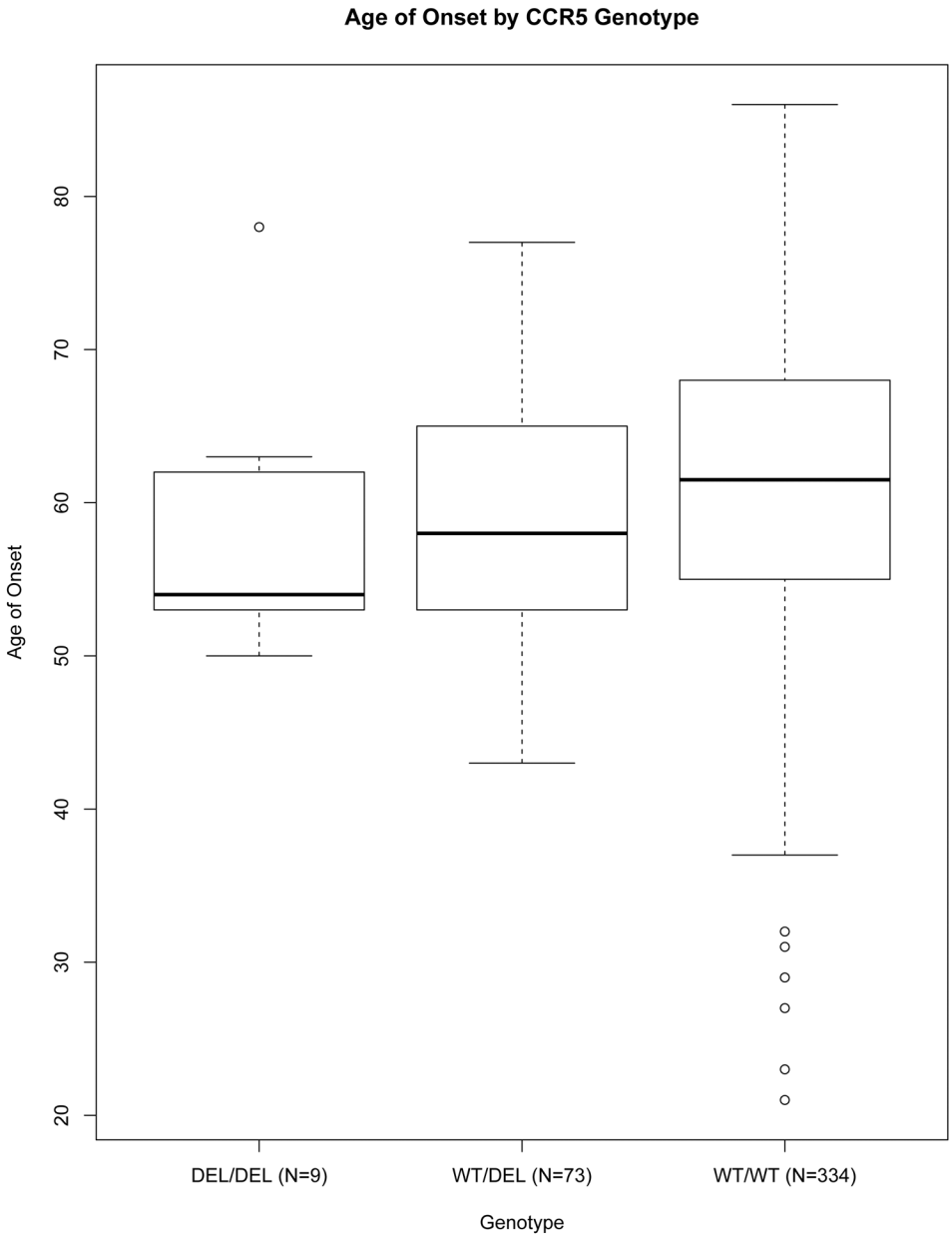

Figure 2: Age of onset by *CCR5*-delta32 genotype for overall neurodegenerative cohort

Age of onset is represented across all patients, shown for *CCR5*-delta32 homozygous (DEL/DEL), *CCR5*-delta32/wild-type heterozygous (WT/DEL), and wild-type homozygous (WT/WT) individuals. 50% of ages of onset by genotype are within each box. Horizontal lines indicate median age of onset. Whiskers indicate highest and lowest ages of onset which are not outliers.

Age of onset was available for a subset of our cohort: AD (179/517, AAO: 62.8), FTD (125/419, AAO: 57.6), MCI (51/279, AAO: 63.0), PSP-S (29/106, AAO: 64.0), CBS (26/158, AAO: 60.1), ALS (6/25, AAO: 51.7).

Table 1:

Allele distribution and demographic characteristics of the 3,457 samples included in this study

Diagnosis	Total	% Female	% European	Age of Onset	*CCR5*-Delta32 Heterozygotes	*CCR5*-Delta32 Homozygotes	% Delta32 Alleles
AD	517	51.1	86.5	62.8 ±10.4	81 (15.7%)	7 (1.4%)	9.2
FTD	419	44.9	93.3	57.6 ±9.9	74 (17.7%)	4 (1%)	9.8
MCI	279	51.3	75.7	63.0 ±8.8	47 (16.8%)	3 (1.1%)	9.5
PSP-S	106	49.1	86.3	64.0 ±7.3	13 (12.3%)	5 (4.7%)	10.8
CBS	79	57.0	87.3	60.1 ±7.7	11 (13.9%)	4 (5.1%)	12.0
ALS	25	40.0	82.6	51.7 ±14.8	5 (20%)	0 (0%)	10.0
Overall	1425	49.3	86.2	61.0 ±10.1	231 (16.2%)	23 (1.6%)	9.7
Controls	2032	56.8	74.3	N/A	336 (16.5%)	25 (1.2%)	9.5

AD: Alzheimer's disease, FTD: frontotemporal dementia, MCI: mild cognitive impairment, PSP-S: progressive supranuclear palsy syndrome, CBS: corticobasal syndrome, ALS: amyotrophic lateral sclerosis. Overall refers to the combined neurodegenerative patient samples.

Age of onset was available in: AD (179/517), FTD (125/419), MCI (51/279), PSP-S (29/106), CBS (26/158), ALS (6/25).

Sex was available in all neurodegenerative disease samples and 1713/2032 controls.

Ancestry percentages were calculated among subset of samples with ancestry available: AD (495/517), FTD (373/419), MCI (259/279), PSP-S (95/106), CBS (71/158), ALS (23/25), Controls (1683/2032)