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ORIGINAL ARTICLE

CCR5-Δ32 polymorphism—a possible protective factor from gait impairment amongst post-stroke patients

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Abstract

Background and purpose: Stroke and small vessel disease cause gait disturbances and falls. The naturally occurring loss-of-function mutation in the C-C chemokine receptor 5 gene (*CCR5*-Δ32) has recently been reported as a protective factor in post-stroke motor and cognitive recovery. We sought to examine whether it also influences gait and balance measures up to 2 years after stroke.

Method: Participants were 575 survivors of first-ever, mild–moderate ischaemic stroke or transient ischaemic attack from the TABASCO prospective study, who underwent a 3 T magnetic resonance imaging at baseline and were examined by a multi-professional team 6, 12 and 24 months after the event, using neurological, neuropsychological and mobility examinations. Gait rhythm and the timing of the gait cycle were measured by force-sensitive insoles. *CCR5*-Δ32 status and gait measures were available for 335 patients.

Results: *CCR5*-Δ32 carriers (16.4%) had higher gait speed and decreased (better) stride and swing time variability 6 and 12 months after the index event compared to non-carriers ($p < 0.01$ for all). The association remained significant after adjustment for age, gender, education, ethnicity and stroke severity.

Conclusions: Significant associations were found between gait measurements and *CCR5*-Δ32 loss-of-function mutation amongst stroke survivors. This is the first study showing that genetic predisposition may predict long-term gait function after ischaemic stroke.

Jeremy Molad and Hen Hallevi contributed equally.

KEYWORDS
CCR5-Δ32 polymorphism, gait, insoles, stroke

INTRODUCTION

Increased step-to-step variability in gait characteristics such as step length, stride time and swing time are associated with adverse outcomes such as mobility limitations and falls amongst older adults, especially after cerebrovascular events [1–3]. Gait variability is altered by aging, disease and injury [4] as well as in individuals with stroke compared to healthy controls and relates to hemiparetic walking performance [5]. Specifically, impairments in swing time and pre-swing variability, increased step length and stride time variability can be indicative of underlying sensorimotor impairments post-stroke [5]. Moreover, gait variability (stride-to-stride fluctuations of gait) is a more sensitive predictor of falls than gait speed [6].

Recently it was reported that the C-C chemokine receptor 5 (*CCR5*), an established factor involved in immune processes and neuromodulation [6], is a promising molecular target for post-stroke recovery. *CCR5* knockdown in rodents was shown to promote motor recovery after stroke and to increase axonal sprouting [7]. In humans, a naturally occurring mutation leading to a non-functional CCR5 protein (in homozygotes) or a partial loss-of-function of CCR5 in heterozygotes (*CCR5*-Δ32, rs333) was found to improve stroke recovery on distinct measures of cognitive and motor functions [7]. However, the impact of *CCR5*-Δ32 on post-stroke motor recovery in human subjects and especially gait measures has not been assessed yet. Therefore, it was sought to examine whether stroke survivors who are carriers of the *CCR5*-Δ32 mutation have better gait performance after a stroke compared with non-carriers.

METHODS

Study population

Participants were consecutive eligible patients from the TABASCO study [8]. Patients included were men and women over 50 years old, admitted within 72 h after a first-ever acute ischaemic stroke or transient ischaemic attack (TIA), with a total score on the National Institutes of Health Stroke Scale (NIHSS) <17 [9]. Exclusion criteria were stroke that resulted from trauma or invasive brain procedures, hemorrhagic stroke, cognitive impairment before the stroke (determined by the short-form of the Informant Questionnaire on Cognitive Decline in the Elderly score ≥3.3) [10], and severe aphasia or disability which made the possibility of follow-up unlikely. Information was collected at the time of recruitment and throughout the study on demographic data, medical history, physical activity, work status, economic status, marital status, functional scores and use of medications, as previously reported [8].

The study individuals were divided into origin groups.

(i) Ashkenazi origin: both parents or grandparents born in a northern/eastern European country, including England, France, Germany, Poland, Russia, the Baltic states, other eastern European countries except Bulgaria.
(ii) Sephardi origin: both parents or grandparents born in Mediterranean or Middle Eastern countries, including Spain, Italy, Greece, Bulgaria, Turkey, Morocco, Algeria, Tunis, Libya, Egypt, Syria, Iraq, Iran, Yemen.
(iii) Mixed origin: one parent from a northern European country and the other from a middle Eastern country.

Study population

A total of 575 consecutive eligible cognitively intact patients at baseline who were admitted to the Department of Emergency Medicine at the Tel-Aviv Sourasky Medical Center between 1 April 2008 and 1 December 2014 within 72 h from the onset of symptoms of TIA or mild–moderate ischaemic stroke were initially evaluated. Of them, *CCR5* genotype was determined in 564 participants; 335 had both *CCR5* genotype and gait and balance data, of whom 238 patients were also evaluated using gait sensors. Gait sensor measurements were performed at 6, 12 and 24 months post index events (see flowchart in Figure S1).

Protocol approvals, registrations and patient consent

This study was registered as ClinicalTrials.gov Identifier NCT01926691 and conducted for 8 years (including follow-up). All participants signed informed consent forms, approved by the local ethics committee (REC Ref: 058-00-TLV). The research was conducted in accordance with the Helsinki Declaration.

Determination of *CCR5* genotype

Genomic DNA was extracted from white blood cells taken from 12 ml of citrated blood and amplified by polymerase chain reaction (PCR), as previously described [11], using 5′-CCTGGCTGTCGTCCATGCTG-3′ and 5′-CTGATCTAGAGCC ATGTGCACAACTCT-3′ as forward and reverse primers. These primers amplify a 735-bp fragment. Following PCR amplification of genomic DNA, the amplified products were digested using EcoRI (New England Biolabs), and the digested products were detected following electrophoresis on a 4% MetaPhor agarose

gel. After restriction, the 735-bp PCR product was cleaved into a common band of 332 bp for both alleles, and a 403-bp product for the wild-type and a 371-bp product for the mutant Δ32 allele. Heterozygous individuals had wild-type and mutant alleles and demonstrated three bands (403, 371, 332 bp) (Figure S2).

Gait characteristics

Subjects walked whilst wearing force-sensitive insoles that measure the foot fall at each gait cycle [12]. Measures included average gait speed, average stride time and average swing time (percent of stride time). Gait stability was quantified by measuring the stride-to-stride variations of the stride time and the swing time, using the coefficient of variation (CV). Higher values of the CV (increased variability) indicate greater instability and have been associated with an increased risk of falls [13].

Gait was tested under two conditions.

(i) Usual walking: participants were instructed to walk along a 20-m path at their comfortable speed for 1 min and were allowed to use assistive devices (canes) as necessary. Two trials were performed and the mean speed was used for analysis.
(ii) Attention-demanding dual task: subjects were asked to perform serial subtractions of 3 from 100 for 1 min whilst walking. Gait speed was determined from the time the subject walked along the 20-m corridor. Two trials were performed and the speed of the second trial was used for analysis [14].

The Timed Up and Go test is a standardized timed performance-based test of gait stability and risk of falls [15]. Subjects were instructed to stand up, walk at a comfortable pace for a distance of 3 m, turn 180°, walk back to the chair and sit down [14]. The second trial was used in the present analyses.

The Berg Balance Scale is a physical performance measure that includes 14 items designed to assess both static and dynamic balance [16]. Gait and balance evaluations were measured at 6, 12 and 24 months after the event by trained assessors.

Off-line analysis of gait

As previously described [17–19], for each leg the time series of the force profile was analyzed by an algorithm that automatically detects heel-strike and the toe-off in each gait cycle. After preprocessing, the following temporal gait parameters were determined: stride time is the time between two consecutive heel-strikes of the same leg; for each walking condition, the mean stride time was calculated for the left and right leg; swing time is the time lapse between toe-off and a consecutive heel-strike of the same leg; for each walking condition, the mean swing time was calculated for the left and right leg. CV was computed for swing and stride time for everyone, according to the formula

$$\text{CV} = (\text{standard deviation} / \text{mean}) \times 100$$

Gait asymmetry was assessed by comparing the swing times performed by one leg with respect to those performed by the other using the formula

$$\text{gait asymmetry} = 100 \times \ln(\text{SSWT} / \text{LSWT})$$

where SSWT and LSWT stand for the mean value of swing time for the leg with the short and long mean swing time, respectively [20].

Baseline and follow-up cognitive assessments

Patients completed a baseline neuropsychological assessment including the Montreal Cognitive Assessment [21] and the NeuroTrax computerized cognitive testing (NeuroTrax Corp.) [22]. These comprehensive neuropsychological evaluations were repeated 6, 12 and 24 months after the stroke: the average of the six index scores (memory, executive functions, visuo-spatial perception, verbal function, attention and motor skills) was computed as the global cognitive score, as previously described [22].

Magnetic resonance imaging (MRI) protocol

Magnetic resonance images were acquired within 7 days of stroke onset on a 3 T GE scanner (GE-Signa EXCITE) using an 8-channel head coil and according to a previously described protocol [8, 23]. All axial slices were prescribed on the same orientation, covering the whole brain, aligned along the fourth ventricle–orbitofrontal orientation. MRI analyses were previously described [23, 24].

Statistical analyses

Continuous variables were analyzed for normality and displayed as mean (±SD) or as a median value with an interquartile range. Comparisons or distributions between categories were assessed using Student's *t* test and the Mann–Whitney *U* test (for NIHSS and the modified Rankin score), as appropriate. Categorical data were analyzed using the Fisher exact test. Missing data were filled in with a multiple imputation method under the "missing at random" assumption. Associations between numeric variables were determined using the Pearson's correlation analysis (coefficient estimate *r*). In order to examine differences in gait trajectories across time the repeated measures approach based on the general linear model was used for comparison of the gait measures of the three observation points between carriers and non-carriers. Statistical differences between longitudinal gait measure curves of the different genotypes were analyzed by a two-way ANOVA, using the Bonferroni correction.

A multiple linear regression model was used to evaluate the relations of the *CCR5*-Δ32 alleles and gait measures at 6, 12 and 24 months post-stroke, adjusted for age, gender, education, ethnicity and stroke severity (admission NIHSS). A p value < 0.05 was considered statistically significant for all analyses. SPSS/WIN (version 27.0, SPSS) software was used for all statistical analyses.

RESULTS

Participants included in the study were slightly younger than those who were not included in the study (66.9 ± 9.7 vs. 68.5 ± 10.3, $p = 0.056$) and had a lower prevalence of diabetes mellitus (25.1% vs. 37.4%, $p = 0.002$) compared to participants not included in the study. A summary of gait characteristics of the participants (335) who had both the *CCR5* genotype and gait measures data is presented in Table 1.

Participants had a mean age of 66.9 (±9.7) years; 63% were male; 280 participants (83.6%) were homozygotes for the *CCR5* wild-type allele (non-carriers), whilst 52 participants (15.5%) were heterozygotes for the *CCR5*-Δ32 variant allele and three patients (0.9%) were homozygotes for the variant allele. Allele frequencies were in Hardy–Weinberg equilibrium. As only a small number ($N = 3$) of participants were *CCR5*-Δ32 homozygotes, all *CCR5*-Δ32 variant allele carriers were pooled together in a *CCR5*-Δ32 carrier group ($N = 55$) and compared to non-carrier homozygotes ($N = 280$).

Stroke etiologies (based on TOAST criteria) were as follows: 131 lacunar strokes (58%), 32 cardioembolic strokes (14.2%), 24 large-artery atherosclerotic strokes (10.6%) and 39 strokes of other or undetermined etiology (17.3%); 109 patients (32.5%) were diagnosed as suffering from TIA.

No differences in *CCR5*-Δ32 distribution were observed across stroke subtypes or between stroke and TIA patients. *CCR5*-Δ32 carriers were more educated but did not differ in cardiovascular risk factors or in the frequency of the apolipoprotein E4 allele compared to non-carriers and had lower C-reactive protein at admission (Table 1). Of the 335 participants, 210 (62.7%) were of Ashkenazi origin, 110 (32.8%) of Sephardi origin and 15 (4.5%) were of mixed origin. 92.7% of the *CCR5*-Δ32 carriers were Ashkenazi in their origin, compared to 56.8% of the non-carriers (the *CCR5*-Δ32 mutation was previously described as more prevalent amongst Ashkenazi Jews [25]). In fact, 52 (24.8%) of the Ashkenazi group were *CCR5*-Δ32 carriers compared to two (1.8%) of Sephardi origin and two (13.3%) of the mixed origin group. The homozygous CCR5 Δ32/Δ32 genotype was prevalent only amongst the Ashkenazi group: 3 (1.4%). *CCR5*-Δ32 carriers had slightly better Berg Balance Scale scores 6 months after the index event, but the difference was not clinically significant (Table 1).

CCR5-Δ32 status, neurological findings at admission and MRI results

CCR5-Δ32 carriers had better neurological scores (lower NIHSS) at admission compared with non-carriers (2 [0–3] vs. 2 [0–4], $p = 0.011$). Both patient groups had relatively low NIHSS scores as only mild to moderate stroke survivors were included in the study, but amongst patients with relatively worse neurological deficit (NIHSS ≥5) there were only a few *CCR5*-Δ32 carriers (4.3% carriers vs. 95.7% non-carriers, $p = 0.024$). No relation was observed between *CCR5*-Δ32 status and lesion side, infarct volume, stroke etiology, markers of small vessel disease or any other MRI finding (Table 1).

CCR5-Δ32 and gait characteristics

Of the included participants, 238 completed gait tests using gait sensors after 6 months, 188 after 12 months and 149 after 24 months. There was no significant difference in gait characteristics between different ethnic groups.

CCR5-Δ32 carriers presented lower (better) stride time variability at 6 and 12 months after the index event compared with non-carriers ($p < 0.001$ for both times), lower swing time variability 6 and 12 months after the index event ($p < 0.001$ for both times), lower (better) swing percentage 6 and 12 months after the index event ($p < 0.001$ for both times) and higher speed 6 months after the index event ($p = 0.011$) (Figure 1). All results remained significant after adjustment for age, gender, education, ethnicity and stroke severity (admission NIHSS) (Table 2). Sub-analysis without the three *CCR5*-Δ32 homozygous subjects resulted in similar outcomes. No significant differences were observed in the frequency of falls after the index event (Table 1). However, this cohort included only mild to moderate stroke or TIA patients, and *CCR5*-Δ32 carriers did not differ significantly in the prevalence of motor weakness at hospital admission (Table 1).

On average, both carriers and non-carriers presented decreased speed and increased stride time variability over time, whilst the non-carrier group presented lower speed and higher stride variability than carriers at all time points and post hoc tests revealed that their scores differed significantly ($p = 0.021$ and $p = 0.003$, respectively, for the linear difference between curves, Figure 1a,c).

No significant differences were observed between the high NIHSS score group (above median) and the low NIHSS score group (below median) in gait characteristics (stride time variability, swing time variability, swing asymmetry) at all time points.

As our cohort included both stroke and TIA patients, a sub-analysis was performed for patients with evidence of acute diffusion-weighted imaging lesion in neuroimaging (evidence of acute stroke), and the results were similar in stride time variability, swing time variability swing percent and swing asymmetry.

CCR5-Δ32 and gait characteristics during dual task

CCR5-Δ32 carriers presented lower (better) stride time variability during the dual task 6 and 12 months after the index event, compared with non-carriers (0.031, 0.003, respectively), lower swing time variability during the dual task 12 months after the index event ($p = 0.005$) and lower swing asymmetry during the dual task 12 months after the index event ($p = 0.001$). All results remain significant after adjustment for age, gender, education, ethnicity and stroke severity (admission NIHSS) (Table 2).

The correlation between stride time variability during the dual task and cognitive performance revealed a significant negative association for carriers and non-carriers. However, *CCR5*-Δ32 non-carriers presented weaker correlation and a less sharp slope (Figure 2) than carriers, namely lower cognitive scores, starting already at low stride time variability.

TABLE 1 Baseline and follow-up characteristics of post-stroke survivors ($n = 335$).

	CCR5-Δ32 non-carriers	*CCR5*-Δ32 carriers	
	$n = 280$	$n = 55$ (52 heterozygote, 3 homozygote)	*p* value
Age, years (SD)	66.4 (9.6)	69.1 (9.9)	0.06
Male gender, *n* (%)	182 (65)	29 (52.7)	0.094
Education, years (SD)	13.2 (3.8)	14.5 (4.2)	**0.026**
Body mass index, kg/m^2 (SD)	27.2 (4.4)	27.5 (3.8)	0.693
Ethnicity, Ashkenazi, *n* (%)	159 (56.8)	51 (92.7)	**<0.001**
Current smokers, *n* (%)	57 (20.4)	8 (14.5)	0.451
Diabetes mellitus, *n* (%)	69 (24.6)	15 (27.3)	0.734
Dyslipidemia, *n* (%)	148 (52.9)	30 (54.5)	0.883
Hypertension, *n* (%)	164 (58.6)	33 (60.6)	0.882
Ischaemic heart disease, *n* (%)	47 (16.8)	13 (23.6)	0.249
Physical activity, min/week	102.4 (169.8)	76.9 (122.6)	0.290
APOE ε4 allele, *n* (%)	52 (18.6)	7 (12.7)	0.434
Admission NIHSS, median (IQR)	2 (0–4)	1 (0–3)	**0.011**
Motor weakness at admission, *n* (%)	122 (43.6)	20 (36.4)	0.185
NIHSS 6 months post-stroke, median (IQR)	0 (0–1)	0 (0–0.5)	**0.048**
Admission modified Rankin score, median (IQR)	1 (0–3)	1 (0–2)	0.607
Modified Rankin score, 6 months post-stroke, median (IQR)	0 (0–1)	0 (0–1)	0.079
TIA (%)	89 (31.8)	20 (36.4)	0.210
CRP at hospital admission, mg/l, median (IQR) (SD)	3.5 (1.2–9.0)	2.7 (0.6–7.3)	**0.042**
Admission Berg Balance Scale	51.9 (10)	53.9 (2.9)	0.056
Admission Timed Up and Go test time, s	9.9 (3.2)	9.6 (2.5)	0.635
Events of falls within 6 months after the index event (%)	49 (17.5)	8 (14.5)	0.680
Events of falls within 6–12 months after the index event (%)	54 (19.3)	8 (14.5)	0.323
Performance-based measures of mobility at 6 months post-stroke ($n = 335$)			
6 months Berg Balance Scale	51.6 (8)	53.9 (3.7)	**<0.001**
6 months Timed Up and Go test time, s	10.7 (4.1)	10.3 (3.6)	0.601
6 months preferred gait speed (m/s)	1.3 (0.4)	1.5 (0.2)	**0.011**
Usual walking measures of gait at 6 months post-stroke ($n = 238$)			
Strong leg swing time CV (%)	6.8 (5.9)	3.9 (2.3)	**<0.001**
Weaker leg swing time CV (%)	4.7 (3.5)	3.5 (1.4)	**0.006**
Weaker leg swing percent CV (%)	4.3 (3.7)	2.7 (1.3)	**<0.001**
Strong leg stride time CV (%)	4.3 (5.2)	2.6 (2)	**0.001**
Weaker leg stride time CV (%)	2.8 (1.5)	2.1 (0.6)	**0.001**
Strong leg stride time during dual task, CV (%)	3.9 (2.3)	3.7 (3.4)	0.604
Weaker leg stride time during dual task, CV (%)	3.0 (1.4)	2.3 (0.9)	**0.031**
Swing asymmetry	0.097 (0.134)	0.081 (0.156)	0.621
Cognitive scores at admission			
Admission MoCA score (SD)	23.4 (3.7)	24.3 (3.3)	0.131
Computerized total cognitive score at admission (SD)	91.8 (14.2)	93.7 (11.9)	0.458
Brain MRI parameters			
Not performed MRI, *n* (%)	61 (21.8)	10 (18.2)	0.550
Infarct type			
No infarct in MRI, *n* (%)	83 (37.9)	15 (33.3)	0.627

TABLE 1 (Continued)

	CCR5-Δ32 non-carriers	*CCR5*-Δ32 carriers	
	n = 280	*n* = 55 (52 heterozygote, 3 homozygote)	*p* value
Cortical infarct, *n* (%)	52 (23.7)	15 (33.3)	0.684
Sub-cortical infarct, *n* (%)	68 (31.1)	11 (24.4)	0.529
Subtentorial infarct, *n* (%)	19 (8.7)	5 (11.1)	0.544
Ischaemic lesion volume, mm^3 (SD)	3280.1 (8720.5)	5557.4 (12669)	0.346
Total WM volume, mm^3 (SD)	446038.7 (62818.5)	440218.8 (72602.2)	0.587
Total GM volume, mm^3 (SD)	580913.7 (60081.3)	564762.2 (66482.4)	0.113
Microbleed lobar yes/no (%)	23 (8.2)	6 (10.9)	0.516
Microbleed deep yes/no (%)	7 (2.5)	2 (3.6)	0.634
Lacunes yes/no (%)	82 (29.3)	19 (34.5)	0.437
Enlarged perivascular spaces yes/no (%)	201 (71.8)	43 (78.2)	0.330

Note: Entries are mean (SD), for numeric variables with normal distribution, or median (IQR), for numeric variables with abnormal distribution, or *n* and % for categorical variables, as indicated. Significant results are shown in bold ($p < 0.05$).

Abbreviations: CRP, C-reactive protein; CV, coefficient of variation; GM, gray matter; IQR, interquartile range; MoCA, Montreal Cognitive Assessment; MRI, magnetic resonance imaging; NIHSS, National Institutes of Health Stroke Scale; SD, standard deviation; TIA, transient ischaemic attack; WM, white matter.

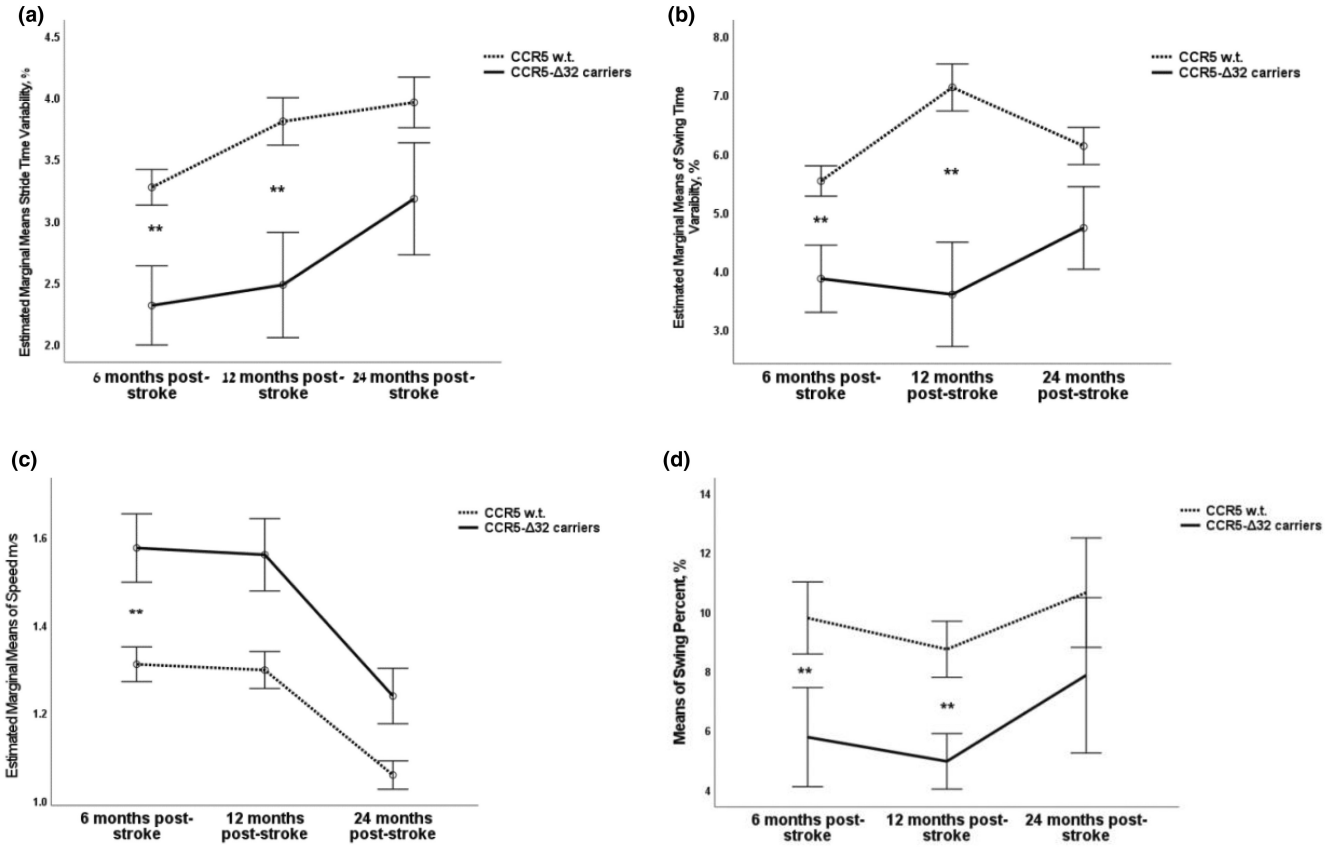

FIGURE 1 General linear model analysis of repeated measures of longitudinal gait measures along the follow-up, comparing *CCR5*-Δ32 carriers to non-carriers.

DISCUSSION

In this study, it is reported for the first time that carriers of a naturally occurring loss-of-function mutation of *CCR5*-Δ32 are associated with certain aspects of better gait 6 and 12 months post-stroke. Our results were consistent across various gait measurements including swing time and stride time, covariance and dual tasking. *CCR5*-Δ32 carriers had higher walking speed, and lower stride and swing

TABLE 2 *CCR5*-Δ32 allele in relation to gait measures post-stroke/TIA, unadjusted (model 1) and adjusted for age, gender, education, ethnicity and admission NIHSS (model 2).

	Model 1			Model 2		
	β	SE	*p*	*β*	SE	*p*
Strong leg swing time CV (variability) (%) at 6 months	**−0.177**	**1.011**	**0.006**	**−0.184**	**1.279**	**0.016**
Strong leg swing time CV (variability) (%) at 12 months	**−0.185**	**1.158**	**0.011**	**−0.181**	**1.403**	**0.036**
Strong leg swing time CV (variability) (%) at 24 months	−0.086	2.141	0.336	–	–	–
Weaker leg swing time CV (variability) (%) at 6 months	−0.135	0.757	0.109	–	–	–
Weaker leg swing time CV (variability) (%) at 12 months	−0.080	0.749	0.370	–	–	–
Weaker leg swing time CV (variability) (%) at 24 months	−0.134	0.534	0.181	–	–	–
Strong leg stride time CV (%) at 6 months	**−0.185**	**0.051**	**0.004**	−0.131	1.135	0910.
Strong leg stride time CV (%) at 12 months	−0.118	0.637	0.106	–	–	–
Strong leg stride time CV (%) at 24 months	−0.154	0.068	0.061	–	–	–
Weaker leg stride time CV (%) at 6 months	**−0.161**	**0.333**	**0.05**	−0.179	0.354	0.055
Weaker leg stride time CV (%) at 12 months	0.078	0.320	0.386	–	–	–
Weaker leg stride time CV (%) at 24 months	−0.138	0.295	0.168	–	–	–
Strong leg stride time during dual task, CV (%) at 6 months	−0.034	0.469	0.604	–	–	–
Strong leg stride time during dual task, CV (%) at 12 months	**−0.161**	**0.591**	**0.028**	−0.158	0.719	0.066
Strong leg stride time during dual task, CV (%) at 24 months	0.004	0.683	0.959	–	–	–
Weaker leg stride time during dual task, CV (%) at 6 months	**−0.184**	**0.312**	**0.031**	**−0.196**	**0.346**	**0.024**
Weaker leg stride time during dual task, CV (%) at 12 months	−0.076	0.515	0.389	–	–	–
Weaker leg stride time during dual task, CV (%) at 24 months	**−0.201**	**0.460**	**0.05**	−0.179	0.396	0.130
Swing asymmetry at 6 months	−0.042	0.032	0.621	–	–	–
Swing asymmetry at 12 months	**−0.232**	**0.022**	**0.009**	**−0.268**	**0.027**	**0.01**
Swing asymmetry at 24 months	−0.077	0.033	0.840	–	–	–

Note: Significant results ($p < 0.05$) are shown in bold.

Abbreviations: CV, coefficient of variation; NIHSS, National Institutes of Health Stroke Scale; SE, standard error; TIA, transient ischaemic attack.

variability of step length and swing time percent 6 and 12 months after the index event compared with non-carriers. In fact, *CCR5*-Δ32 carriers had gait characteristics that were similar to those of elderly healthy subjects [26–28]. Disease states, including stroke, can affect the variability of the spatiotemporal characteristics of gait [4, 5, 29]. Previous studies examining gait measures in older adults have found decreased walking speed as well as higher swing and stride variability to be the most associated with risk for mobility limitations [29, 30].

Gait changes predict dementia as much as 6–10 years later and may also precede the onset of even mild cognitive impairment [31], which may occur as much as 12 years before the clinical presentation of cognitive changes [32]. Moreover, the combination of motor and cognitive changes is apparently a stronger predictor of dementia than cognitive changes alone [33]. Whilst the mechanisms of these motor–cognitive interactions are not fully understood, subclinical pathological changes caused by cerebrovascular disease may play a role.

Increased stride time variability amongst the non-carriers may be a prognostic marker of falls and cognitive deterioration. Several studies performed in recent years by our group and others have shown that *CCR5*-Δ32 is negatively associated with post-stroke cognitive decline [7] and post-stroke depression [34] in human subjects and with better post-stroke motor function in mice [7]. Indeed, in our results, *CCR5*-Δ32 non-carriers presented higher stride time

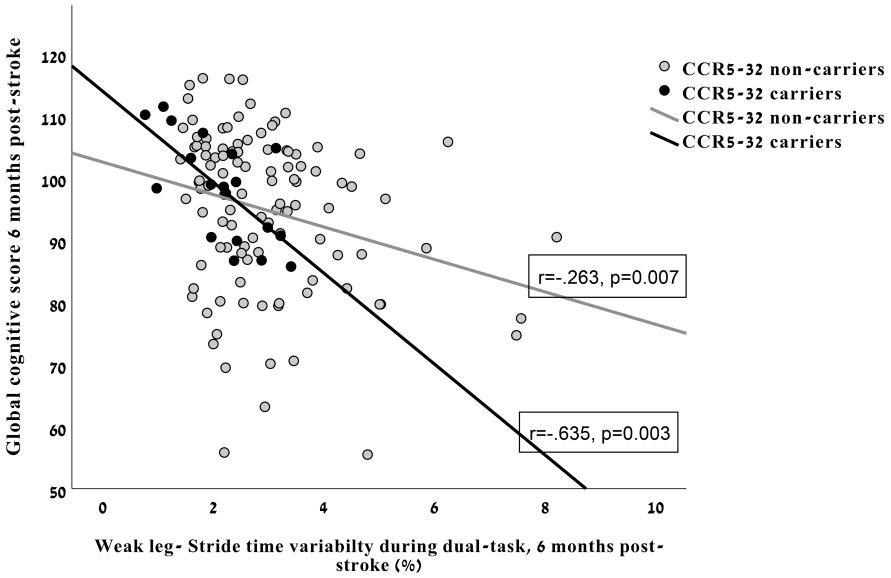

FIGURE 2 Association between stride time variability during the dual task 6 months after the index event and cognitive performance, according to CCR5 genotype.

variability and lower cognitive scores than carriers, which may imply motor–cognitive interactions.

Importantly, *CCR5*-Δ32 carriers and non-carriers in our cohort had similar levels of cardiovascular risk factor, and similar stroke volumes and locations, cerebral small vessel disease markers on MRI and brain volume measurements. Furthermore, as our cohort included both mild to moderate stroke patients and TIA patients, a secondary analysis was performed including only patients with evident sub-acute ischaemic stroke in MRI, showing similar results. This emphasizes the direct influence of CCR5 on stroke recovery, which is beyond its association with stroke severity since both variables were included in the prediction model.

CCR5 is widely expressed in the central nervous system and in involved in neuronal differentiation, brain development and learning. CCR5 expression is upregulated in neurons after stroke [7]. The association between impaired CCR5 activity and recovery from brain injury may be attributed to several possible mechanisms of action. *CCR5*-Δ32 may reduce the inflammatory reactions that appear after the ischaemic insult and therefore reduce secondary tissue damage. Previous primate studies have shown that CCR5 blockade attenuates the activation of glial cells and neuroinflammation, maintains the integrity of the endothelial monolayer and reduces the infiltration of T cells to the brain. CCR5 blockade was also found to suppress T-lymphocyte chemotactic activity and modify the production of proinflammatory cytokines in vitro [35, 36]. Indeed, *CCR5*-Δ32 carriers in our cohort had lower C-reactive protein levels at admission, reflecting a decreased inflammatory profile. CCR5 blockage was also found to enhance tissue preservation in a model of closed head injury in mice, in various brain regions including the cortex, hippocampus, sub-cortical areas and corpus callosum [6, 7]. Another possible mechanism is related to CCR5 impact on neuromodulation and improved synaptic plasticity. CCR5 blockade may prevent the loss of synaptic connections after stroke or promote the formation of new connections. This is probably mediated by CREB and dual leucine zipper kinase signaling, which mediate injury signals, dendritic spine morphogenesis, axonal regeneration and motor recovery [6, 7, 37]. It was previously shown that CCR5 knockdown in rodents stabilized dendritic spines after experimental stroke in tissue adjacent to the infarct and induced a remarkable degree of axonal sprouting in the bihemispheric or callosal connections of premotor cortex after experimental stroke [7]. Moreover, CCR5 blockade was associated with activation of synaptic signaling molecules including CREB and *N*-methyl-D-aspartate subunit 1 [38].

In recent years, several preclinical experiments have found that treatment with Maraviroc, a CCR5 antagonist, is associated with neuroprotection and improved brain repair and recovery after experimental stroke or traumatic brain injury [7]. Maraviroc was also reported to improve cognitive function in chronic human immunodeficiency virus (HIV) infected patients experiencing HIV-associated dementia [39, 40]. Recently, our group has initiated a phase 2 clinical trial in order to examine the safety and efficacy of Maraviroc in post-stroke cognitive impairment [41]. The result of the current study further supports the potential effect of Maraviroc in stroke recovery.

The difference in the *CCR5*-Δ32 frequency between Ashkenazi and Sephardi Jews corresponds to the difference in *CCR5*-Δ32 prevalence between north European and Mediterranean populations in general [42, 43]. Additionally, founder effect and genetic drift are proposed to explain the elevated values observed in Ashkenazi Jews [44].

The strengths of the current study include the objective gait assessment, with repeated measures, the prospective follow-up with comprehensive data on participants' clinical status and macrostructural MRI measures. The combination of these factors made this study unique. Other strengths include the advantage of a cohort that consisted of a high percentage of Ashkenazi Jews, more of whom carry the *CCR5*-Δ32 mutation than other populations.

Limitations of our study include the exclusion of patients who had severe stroke, who may potentially suffer from higher motor limitations. Thus, our findings cannot be generalized to this sub-group of patients and further research amongst them is warranted. However,

the significant findings in our mild to moderate stroke cohort support our hypothesis, as well as the finding that amongst patients with greater neurological deficits (higher NIHSS) there were only a few *CCR5*-Δ32 carriers. Also, a control age- and gender-matched group without stroke history would add important data to our results.

The fact that 57% of our population were Ashkenazi in origin may limit the results of this study to this demographic. However, the association of the *CCR5*-Δ32 status with gait measures remained significant after adjustment for ethnicity.

Study limitations derive mainly from the fact that only 71% of the cohort had gait measurements using insoles. The use of insoles was added to the TABASCO protocol as an exploratory measure after the beginning of the trial, and the recruitment of patients included in the current analysis was mostly consecutive. Furthermore, no differences were found in the baseline characteristics, cognitive scores and *CCR5*-Δ32 allele rates between patients who underwent gait assessment using insoles and those without.

Lastly, even though the loss to follow-up was relatively modest, there was probably some selection bias. Multiple imputations were used to address this problem and provide an estimate of probable gait measures in patients lost to follow-up. Many predictor variables were used, demonstrating consistent results. Also, no differences were observed in baseline characteristics between participants included and not included in the study, besides a slight difference in age and the prevalence of diabetes mellitus. Thus, it seemed reasonable to assume that data were missing at random. Under the assumption of missing at random, valid inferences may be obtained by applying the multiple imputation technique. Analysis of the observed dataset (including imputed values) revealed the same trend of results for gait and cognitive scores.

CONCLUSION

In conclusion, *CCR5*-Δ32 allele was associated with better gait measurements in a prospective cohort of stroke survivors. This finding is in line with previous studies which described an association between *CCR5*-Δ32 allele and stroke recovery, supporting *CCR5*-Δ32 as a promising molecular target for intervention to improve stroke recovery.

AUTHOR CONTRIBUTIONS

JM: Conception, acquisition, execution, analysis and interpretation of the data, writing—original draft. HH: Conception, acquisition, execution, writing—original draft. ES: Acquisition, revising original draft. OR: Acquisition, revising original draft. NMB: Conception, acquisition, execution. OT: Acquisition, revising original draft. NG: Conception, methodology, revising original draft. JH: Conception, methodology, revising original draft. AM: Conception, methodology, revising original draft. EBA: Conception, secure funding, acquisition, execution, analysis and interpretation of the data, statistical analysis, writing—original draft, editing revised draft.

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CONFLICT OF INTEREST

EBA, HH, OT and the Tel-Aviv Sourasky Medical Center have a pending patent application filed #62/978,324. Specific aspects of manuscript covered in patent application: using CCR5 antagonists for treating post-stroke cognitive impairment. JM, ES, OR, NMB, NG, JH and AM declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Hausdorff JM, Rios DA, Edelberg HK. Gait variability and fall risk in community-living older adults: a 1-year prospective study. *Arch Phys Med Rehabil*. 2001;82:1050-1056.
2. Brach JS, Studenski SA, Perera S, VanSwearingen JM, Newman AB. Gait variability and the risk of incident mobility disability in community-dwelling older adults. *J Gerontol A Biol Sci Med Sci*. 2007;62:983-988.
3. Brach JS, Berthold R, Craik R, VanSwearingen JM, Newman AB. Gait variability in community-dwelling older adults. *J Am Geriatr Soc*. 2001;49:1646-1650.
4. Hausdorff JM. Gait dynamics, fractals and falls: finding meaning in the stride-to-stride fluctuations of human walking. *Hum Mov Sci*. 2007;26:555-589.
5. Balasubramanian CK, Neptune RR, Kautz SA. Variability in spatiotemporal step characteristics and its relationship to walking performance post-stroke. *Gait Posture*. 2009;29(3):408-414.
6. Zhou M, Greenhill S, Huang S, et al. CCR5 is a suppressor for cortical plasticity and hippocampal learning and memory. *Elife*. 2016;5:e20985.
7. Joy MT, Ben Assayag E, Shabashov-Stone D, et al. CCR5 is a therapeutic target for recovery after stroke and traumatic brain injury. *Cell*. 2019;176(5):1143-1157.e13.
8. Ben Assayag E, Korczyn AD, Giladi N, et al. Predictors for poststroke outcomes: the Tel Aviv Brain Acute Stroke Cohort (TABASCO) study protocol. *Int J Stroke*. 2012;7(4):341-347.
9. Brott T, Adams HP Jr, Olinger CP, et al. Measurements of acute cerebral infarction: a clinical examination scale. *Stroke*. 1989;20(7):864-870.
10. Jorm AF. A short form of the informant questionnaire on cognitive decline in the elderly (IQCODE): development and cross-validation. *Psychol Med*. 1994;24(1):145-153.
11. Lucotte G. Frequencies of the CC chemokine receptor 5 delta 32 allele in various populations of defined racial background. *Biomed Pharmacother*. 1997;51(10):469-473. doi:10.1016/s0753-3322(97)82328-1

12. Hausdorff JM, Gruendlinger L, Scollins L, O'Herron S, Tarsy D. Deep brain stimulation effects on gait variability in Parkinson's disease. *Mov Disord*. 2009;24(11):1688-1692.
13. Hausdorff JM. Gait variability: methods, modeling and meaning. *J Neuroeng Rehabil*. 2005;20(2):19.
14. Ben Assayag E, Shenhar-Tsarfaty S, Korczyn AD, et al. Gait measures as predictors of poststroke cognitive function: evidence from the TABASCO study. *Stroke*. 2015;46(4):1077-1083.
15. Podsiadlo D, Richardson S. The timed "Up & go": a test of basic functional mobility for frail elderly persons. *J Am Geriatr Soc*. 1991;39(2):142-148.
16. Berg K, Wood-Dauphinee S, Williams JI. The balance scale: reliability assessment with elderly residents and patients with an acute stroke. *Scand J Rehabil Med*. 1995;27:27-36.
17. Hausdorff JM, Cudkowicz ME, Firtion R, Wei JY, Goldberger AL. Gait variability and basal ganglia disorders: stride-to-stride variations of gait cycle timing in Parkinson's disease and Huntington's disease. *Mov Disord*. 1998;13:428-437.
18. Hausdorff JM, Schaafsma JD, Balash Y, Bartels AL, Gurevich T, Giladi N. Impaired regulation of stride variability in Parkinson's disease subjects with freezing of gait. *Exp Brain Res*. 2003;149:187-194.
19. Plotnik M, Bartsch RP, Zeev A, Giladi N, Hausdorff JM. Effects of walking speed on asymmetry and bilateral coordination of gait. *Gait Posture*. 2013;38(4):864-869.
20. Plotnik M, Giladi N, Balash Y, Peretz C, Hausdorff JM. Is freezing of gait in Parkinson's disease related to asymmetric motor function? *Ann Neurol*. 2005;57(5):656-663.
21. Nasreddine ZS, Phillips NA, Bedirian V, et al. The Montreal cognitive assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc*. 2005;53(4):695-699.
22. Doniger GM, Dwolatzky T, Zucker DM, et al. Computerized cognitive testing battery identifies mild cognitive impairment and mild dementia even in the presence of depressive symptoms. *Am J Alzheimers Dis Other Demen*. 2006;21(1):28-36.
23. Tene O, Shenhar-Tsarfaty S, Korczyn AD, et al. Depressive symptoms following stroke and transient ischemic attack: is it time for a more intensive treatment approach? Results from the TABASCO cohort study. *J Clin Psychiatry*. 2016;77(5):673-680.
24. Molad J, Hallevi H, Korczyn AD, et al. Vascular and neurodegenerative markers for the prediction of post-stroke cognitive impairment: results from the TABASCO study. *J Alzheimers Dis*. 2019;70(3):889-898.
25. Maayan S, Zhang L, Shinar E, et al. Evidence for recent selection of the CCR5-delta 32 deletion from differences in its frequency between Ashkenazi and Sephardi Jews. *Genes Immun*. 2000;1(6):358-361.
26. Jacobs D, Farid L, Ferré S, Herraez K, Gracies JM, Hutin E. Evaluation of the validity and reliability of connected insoles to measure gait parameters in healthy adults. *Sensors (Basel)*. 2021;21(19):6543.
27. Wuehr M, Nusser E, Krafczyk S, et al. Noise-enhanced vestibular input improves dynamic walking stability in healthy subjects. *Brain Stimul*. 2016;9(1):109-116.
28. Hausdorff JM, Hillel I, Shustak S, et al. Everyday stepping quantity and quality among older adult fallers with and without mild cognitive impairment: initial evidence for new motor markers of cognitive deficits? *J Gerontol A Biol Sci Med Sci*. 2018;73(8):1078-1082.
29. James EG, Conatser P, Karabulut M, et al. Walking speed affects gait coordination and variability among older adults with and without mobility limitations. *Arch Phys Med Rehabil*. 2020;101(8):1377-1382.
30. Speiser JL, Callahan KE, Ip EH, et al. Predicting future mobility limitation in older adults: a machine learning analysis of health ABC study data. *J Gerontol A Biol Sci Med Sci*. 2022;77(5):1072-1078.
31. Verghese J, Lipton RB, Hall CB, Kuslansky G, Katz MJ, Buschke H. Abnormality of gait as a predictor of non-Alzheimer's dementia. *N Engl J Med*. 2002;347:1761-1768.
32. Buracchio T, Dodge HH, Howieson D, Wasserman D, Kaye J. The trajectory of gait speed preceding mild cognitive impairment. *Arch Neurol*. 2010;67:980-986.
33. Verghese J, Wang C, Lipton RB, Holtzer R. Motoric cognitive risk syndrome and the risk of dementia. *J Gerontol A Biol Sci Med Sci*. 2013;68:412-418.
34. Tene O, Hallevi H, Molad J, et al. CCR5-Δ32 polymorphism: a possible protective factor for post-stroke depressive symptoms. *J Psychiatry Neurosci*. 2021;46(4):E431-E440.
35. Yuan J, Ren HY, Shi YJ, Liu W. In vitro immunological effects of blocking CCR5 on T cells. *Inflammation*. 2015;38(2):902-910.
36. Mondal S, Rangasamy SB, Roy A, Dasarathy S, Kordower JH, Pahan K. Low-dose Maraviroc, an antiretroviral drug, attenuates the infiltration of T cells into the central nervous system and protects the nigrostriatum in hemiparkinsonian monkeys. *J Immunol*. 2019;202:3412-3422.
37. Moore DL, Goldberg JL. Multiple transcription factor families regulate axon growth and regeneration. *Dev Neurobiol*. 2011;71(12):1186-1211.
38. Liraz-Zaltsman S, Friedman-Levi Y, Shabashov-Stone D, et al. Chemokine receptors CC chemokine receptor 5 and C-X-C motif chemokine receptor 4 are new therapeutic targets for brain recovery after traumatic brain injury. *J Neurotrauma*. 2021;38(14):2003-2017.
39. Gates TM, Cysique LA, Siefried KJ, Chaganti J, Moffat KJ, Brew BJ. Maraviroc-intensified combined antiretroviral therapy improves cognition in virally suppressed HIV-associated neurocognitive disorder. *Aids*. 2016;30(4):591-600.
40. Ndhlovu LC, Umaki T, Chew GM, et al. Treatment intensification with Maraviroc (CCR5 antagonist) leads to declines in CD16-expressing monocytes in cART-suppressed chronic HIV-infected subjects and is associated with improvements in neurocognitive test performance: implications for HIV-associated neurocognitive disease (HAND). *J Neuroyirol*. 2014;20(6):571-582.
41. Ben Assayag E, Molad J, Seyman E, et al. Preventing post-stroke dementia. The MARCH trial. Protocol and statistical analysis plan of a randomized clinical trial testing the safety and efficacy of Maraviroc in post-stroke cognitive impairment. *Eur Stroke J*. 2022;7(3):314-322.
42. Libert F, Cochaux P, Beckman G, et al. The DCCR5 mutation conferring protection against HIV1 in Caucasian populations has a single and recent origin in northeastern Europe. *Human Mol Genet*. 1998;7:399-406.
43. Stephens JC, Reich DE, Goldstein DB, et al. Dating the origin of the CCR5-D32 AIDS-resistance allele by the coalescence of haplotypes. *Am J Hum Genet*. 1998;62:1507-1515.
44. Lucotte G, Smets P. CCR5-delta32 allele frequencies in Ashkenazi Jews. *Genet Test*. 2003;7(4):333-337.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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