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Abstract

Background: Isolated, subtle neurological abnormalities (ISNA) are commonly seen in aging, and have been related to cerebral Small Vessel Disease (SVD) and subcortical atrophy in neurologically and cognitively healthy aging subjects.

Objective: To investigate the frequency of ISNA in different MCI types, and to evaluate for each MCI type the cross-sectional relation between ISNA and white matter hyperintensities (WMH), lacunes, caudate atrophy, and ventricular enlargement.

Methods: 1,250 subjects with different MCI types were included in the analysis, and underwent brain MRI. WMH were assessed through two visual rating scales. Lacunes were also rated. Atrophy of the caudate nuclei and ventricular enlargement were assessed through the bicaudate ratio (BCr) and the lateral ventricles to brain ratio (LVBr), respectively. Apolipoprotein E (APOE) genotypes were also assessed. The routine neurological examination was used to evaluate ISNA that were clustered as central-based signs, cerebellar-based signs, and primitive reflexes. The items of Part-III of the Unified Parkinson's Disease Rating Scale were used to evaluate ISNA that were clustered as mild parkinsonian signs. Associations of ISNA with imaging findings was determined through logistic regression analysis.

Results**:** The ISNA increase with the age, are present in all MCI types, particularly in those multiple domains, and carrying the APOE ε4 allele, and are associated with WMH, lacunes, BCr, and LVBr.

Conclusion: This study demonstrates that cortical and subcortical vascular and atrophic processes contribute to ISNA. Long prospective population-based studies are needed to disentangle the role of ISNA in the conversion from MCI to dementia.

Keywords: Isolated, subtle, neurological abnormalities, Mild cognitive impairment types, White matter hyperintensities, Lacunes, Caudate atrophy, Global cerebral atrophy

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INTRODUCTION

Mild cognitive impairment (MCI) is a term that refers to a condition in which an essentially spared global cognition, and normal or slightly impaired activities of daily living coexist with a mild decline of cognitive functions greater than that expected for age and education.[1,2] Four MCI phenotypes have been recognized[2] as follows: amnestic MCI single domain (a-MCI), amnestic MCI multiple domain (a-MCImd), nonamnestic MCI single domain (na-MCI), and nonamnestic MCI multiple domain (na-MCImd). Extracellular deposition of β-amyloid (Aβ) peptide, intracellular deposition of hyper-phosphorilated tau protein, and atrophy of frontal, parietal, and medial temporal cortices, *i.e.* neurodegeneration of AD signature cortical regions,[3] are the key elements of the pathophysiology of Alzheimer disease (AD),[4] and MCI due to AD.[5] The prevalence of Aβ positivity among subjects with MCI increases from age 50 to 90 years from 27% to 71%; nonamnestic MCI types have lower prevalence estimates of Aβ positivity than amnestic MCI types, but higher than subjects cognitively normal (CN), and both amnestic and nonamnestic MCI are at increased risk for AD.[6] Concerning tau, MCI and AD individuals have tau accumulation in the basal and mild-temporal, retrosplenial, posterior cingulate, and enthorinal regions greater than CN individuals Aβ positive.[7]

The observation that 21,5% and 35% of individuals with amnestic and nonamnestic MCI types, respectively, are Aβ negative,[8] has suggested that these individuals are not on the AD pathway, and that vascular pathology may be one of the possible non-AD causes of MCI. Among vascular pathology, cerebral small vessel disease (SVD) plays a pivotal role. SVD affects the smallest cerebral small vessels, increases throughout the lifespan,[9] and contributes to the risk of MCI,[10] and dementia.[11] White matter hyperintensities (WMH), lacunes, small subcortical infarcts, microbleeds, enlarged perivascular spaces, and central atrophy are the imaging markers of SVD.[12] Lacunes[13] and WMH[14] disrupting locally the structural integrity of white matter induce thinning of the connected cortical regions through Wallerian degeneration. Lacunes are associated with widespread cortical thinning, atrophy in multiple subcortical structures, and ventricular enlargement.[15] In subjects with MCI, expansion of the lateral ventricles is associated with atrophy of frontal, parietal, and temporal regions affected by AD.[16] Furthermore, age *per se* is associated with atrophy of the cerebellum, striatum, and prefrontal, parietal, and temporal association cortices.[17] The pattern of cortical atrophy induced by severe WMH overlaps substantially with the patterns of age-related cortical atrophy and of AD-related cortical atrophy.[18] Since cortical

atrophy induced by WMH drives cognitive decline,[19] age and SVD may contribute to the onset of cognitive decline through the overlapping atrophy of cortical regions vulnerable to AD pathology.

Cerebral SVD, Aβ, tau, and atrophic changes fragmenting over time brain networks into disconnected parts not only contribute to cognitive decline, but also contribute to the presentation of a wide range of neurological signs. We have shown that neurologically and cognitively healthy (NCH) aging subjects frequently present at the routine neurological examination isolated, subtle, neurological abnormalities (ISNA) which do not have any immediate diagnostic relevance, cannot be attributed to any definite, overt neurological disease, are associated with atrophy of the caudate nuclei, and with parietal WMH and lacunes, and probably constitute a red flag for future cognitive decline given that they show poor performance in test evaluating global cognition, executive function, and language.[20] Past reports on neurological signs in MCI have primarily focused on extrapyramidal features,[21,22] while reports on signs others than extrapyramidal are sparse. Therefore, the aims of our study are: 1) to investigate the prevalence of ISNA in the 4 MCI types; 2) to verify whether in the individual MCI types the probability of having ISNA is differently associated with the topographical location of WMH and lacunes, periventricular WMH (WMH-PV), APOE ε4 allele, and with two linear measures of central atrophy,[12] *i.e.* the bicaudate ratio (BCr) as proxy of subcortical atrophy, and the lateral ventricles to brain ratio (LVBr) as proxy of global brain atrophy. In the present paper, the terms "adult", "elderly", "old", and "oldest-old" will be used to indicate people aged 45-64, 65-74, 75-84, and >85 years respectively.

METHODS

Participants

Data were used from the Cognitive Impairment through Aging (CogItA) study, a hospital-based prospective study focused on normal and pathological aging in middle-aged and older individuals launched in January, 2000. CogItA's participants were outpatients self-referred or referred by general practitioners for neurological and/or cognitive screenings to the clinics of the Department of Neurology and Cognitive Disorders of the teaching Hospital (AOUP "P.Giaccone") of the School of Medicine of the University of Palermo, Italy. Details of the inclusion and exclusion criteria of the CogItA study have been reported elsewhere.[20] Informed consent was obtained from all participants and relatives. The study was approved by the University Hospital ethics committee, and complies with the declaration of Helsinki.

According to the published criteria,[1,2] CogItA participants with preserved global cognition at the Mini-Mental State examination (MMSE score ≥ 23,74),[23] subjective cognitive concerns, objective impairment in one or more cognitive domains, Clinical Dementia Rating (CDR) = 0.5),[24] no impaired or minimally impaired functional status on the activities of daily living (ADL)[25] and the instrumental activities of daily living (IADL) [26] scales, and no dementia were classified as MCI and categorized as a-MCI, a-MCImd, na-MCI, and na-MCImd. MCI subjects included in the present paper were stroke-free first ever diagnosed cases (n=1,250) aged 45-95 years (mean age = 70,52 ± 9,41 years), who remained in the MCI status for at least 3 years (mean follow-up = 64,98 ± 28,94 months). During this period, some of these subjects changed their MCI typology, but in the present paper first-ever MCI diagnoses were considered. Subjects who during the follow-up reverted to normal cognition, or converted to dementias different from AD and Vascular Dementia (VaD) were not considered.

Baseline Clinical Assessment

Participants to the present study underwent an extensive assessment of variables such as demographics, medical history, laboratory tests, neurological, and functional examinations, cognitive testing, carotid ultrasonography, and brain magnetic resonance imaging (MRI). Many vascular risk factors (VRF) and vascular diseases (VD) were considered and assessed as reported elsewhere.[20,29] Since multiple VRF and VD often coexist, we created the VRF and VD summary scores indicating for each participant the sum of the individual VRF and VD that were concurrently present. APOE genotypes were determined by using standard methods.[30] Participants with at least 1 APOE ε4 allele were classified as APOE ε4 carriers.

Assessment of ISNA

All participants underwent a standardised neurological examination reflecting that routinely performed in the clinical practice. Subjects presenting at baseline or during follow-up meaningful neurological signs such as visual field defects, language deficits, cranial nerves deficits, hemimotor and hemisensory dysfunction, brachial or crural weakness, brachial or crural sensory dysfunction, Babinski sign, spastic rigidity, and hemiplegic gait were excluded. The ISNA evaluated were: 1) mild dysphagia, 2) slurred speech, 3) central facial weakness, 4) mixed rigidity, *i.e.* a condition in which spastic and plastic rigidity coexist; 5) hyperreflexia (bilateral increased deep tendon reflexes), 6) reflex asymmetry, 7)

tremor (resting tremor, and postural/kinetic tremor), 8) plastic rigidity, 9) bradykinesia, 10) gait/balance/axial dysfunction, 11) dysmetria, 12) atactic type gait defined as a gait pattern broadly indicative of cerebellar involvement, and 13) primitive reflexes (PR) *i.e.* glabellar tap, snout, palmomental, grasping, and sucking reflexes.[31,32] To evaluate tremor, rigidity, bradykinesia, and gait/balance/axial dysfunction, collectively called Mild Parkinsonian Signs (MPS),[33] the items of motor section of the Unified Parkinson's Disease Rating Scale (UPDRS)[34] were used, and were considered present when any one of the following condition was met: (1) two or more items with a score of 1; or (2) one item with a score ≥ 2.[21] The ISNA were dichotomised as absent (score = 0) or present (score = 1), and were clustered into four categories as following: central-based signs (Cs) (signs 1-6), Mild Parkinsonian Signs (MPS) (signs 7-10), cerebellar-based signs (CLs) (signs 11-12), and PR (signs 13). We defined the presence of Cs, MPS, CLs, and PR as the presence of at least one sign within those included in each of these clusters. Accordingly, subjects were divided into subjects without ISNA (ISNA-), and with ISNA (ISNA+) if at least one sign within the above clusters was present. The neurological examination of each participant was always performed by two neurologists blinded to the patients history and neuroimaging. The interrater reliability assessed over time in random samples showed always excellent agreement with weighted Cohen's kappa ranging between 0.88 and 0.91 ($p< 0.001$).

Functional and Neuropsychological Assessments

The functional status of participants was assessed through the ADL and the IADL scales. Cognitive functions were assessed using an extensive neuropsychological battery as reported elsewhere,[20,35] including the MMSE as test of global cognition, and 12 tests to evaluate memory, attention, executive function, language, constructional ability, and visuospatial skill. Impaired cognitive domains were identified using a cut-off of 1,5 standard deviation (SD)[2] below the Italian normative data adjusted for age-, sex-, and education.[36]

Carotid Ultrasonography and Imaging Assessments

Intimal-medial thickness (IMT), and stenosis of internal carotid arteries (SICA) were assessed as reported elsewhere.[20] Participants had brain MRI on a 1,5T scanner (GE Signa HDxt, Milwaukee, WI, USA). Details of the image acquisition protocol have been published previously.[20] The BCr and the LVBr were calculated as reported elsewhere.[20,35] WMH, and lacunes were assessed according to the

published criteria.[12] The Wahlund scale (range 0-3) [37] was used to obtain the scores of WMH of the frontal, parieto-occipital, and temporal areas (WMH-SC), infratentorial (WMH-INF), basal ganglia (WMH-BG), and the WMH total score (WMH-T). To define the WMH status, a cut-off score ≥2 in at least one of the above regions was used. WMH-PV were evaluated with the Fazekas scale (range 0-3),[38] and a cut-off score ≥2 was used. Lacunes were assessed topographically according to the Wahlund regions used to score WMH, and categorized as lacunes-SC, lacunes-BG, lacunes-INF, and lacunes-T. A cut-off score ≥2 in at least one of the Wahlund regions was used to define the status of lacunes. Subjects having WMH and/or lacunes with a score ≥2 in at least one of the Wahlund scale topographical locations were categorized as SVD positive (SVD+), and those having WMH and/or lacunes with a score ≤1 were categorized as SVD negative (SVD-).

Statistical Analysis

Descriptive statistics (percentages, mean and SD, median and IQR) were used to summarise data. Continuous variables were compared between subjects ISNA- and ISNA+ by using one-way analysis of variance (ANOVA), and differences were tested with a post hoc F-test. Categorical variables were evaluated by contingency tables, and the hypotheses of independence were tested with $\chi 2$ test. Logistic ridge regression models[39] were used to evaluate for each MCI type the risk of having ISNA covarying for age, sex, years of education, and the variables found significant in the univariate analysis. In general, ridge regression method is the most applied solution for addressing problems of multicollinearity.[40] It implies adding a small positive constant (λ), *i.e.* the ridge parameter, to the main diagonal elements of the information matrix. The ridge parameter was selected using likelihood cross-validation.[41] IMT, BCr, and LVBr values were scaled to work on percentage of increments. All tests were two-tailed, and statistical significance was set at $p \leq 0.05$. Results are presented as odds ratios (ORs) with 95% confidence interval (95% CI). All analyses were performed using R statistical software (version 3.5.1; The R Foundation for Statistical Computing).

RESULTS

The demographic characteristics of the MCI types are shown in Table 1. In almost all MCI types, the majority of subjects were elderly followed by old, adult, and oldest-old individuals. The MCI types

single domain were more common among the adults, while those multiple domain were more common within the elderly, old, and oldest old participants. In each age class, the mean age didn't vary significantly among the various MCI types. Since the oldest old subjects were few, subsequent analysis was conducted pooling the old and oldest old classes in the class of old-oldest old.

Within the sample, 175 (14.0%) subjects didn't show any of the selected ISNA, while 1,075 (86.0%) presented at least one ISNA (Table 2). Overall, subjects ISNA- were more common among the MCI types single domain, and subjects ISNA+ were more common among those multiple domain. Primitive reflexes were the most frequent ISNA, followed by ISNA central-based, and mild parkinsonian signs, while ISNA cerebellar-based were the rarest. PR were exhibited by 864 subjects (69.1%) of the sample, and were more common among the MCI types multiple domain than those single domain. In all MCI types, snout reflex was the most common PR followed by glabellar tap, and palmomental reflex, while grasping and sucking reflexes were the rarest. Snout and palmomental reflexes were more common among the MCI types multiple domain than those single domain, while glabellar tap was more common among the amnestic MCI types than the nonamnestic types. The mean number of PR was greater among the amnestic MCI types than the nonamnestic types. Central-based signs were exhibited by 645 subjects (51,6%) of the sample. Subjects with Cs were more common among the nonamnestic MCI types than the amnestic types. In all MCI types, reflex asymmetry was the most common Cs, followed by bilateral hyperreflexia, central facial weakness, mixed rigidity, slurred speech, and dysphagia. Reflex asymmetry, bilateral hyperreflexia, and central facial weakness were more common among the nonamnestic MCI types than the amnestic types, while the frequency of the other Cs didn't vary among the 4 MCI types. The mean number of Cs was greater among the nonamnestic MCI types than the amnestic types. Mild Parkinsonian Signs were found in 46,2% (n=578) of the sample. In all MCI types, bradykinesia was the most common MPS, followed by gait/balance/axial dysfunction, and tremor, while rigidity was the rarest. Individual MPS were more common among the MCI types multiple domain than the MCI single domain. The mean number of MPS was greater in the former than in the latter. Cerebellar-based signs were the rarest ISNA encountered. Dysmetria was the most common CLs, and the atactic type gait was the rarest. The frequency of dysmetria didn't vary among the 4 MCI types, while the atactic type gait was more common among the a-MCImd than the other MCI types. The mean number of CLs didn't vary among the MCI types.

ISNA increased with age reaching a peak in the old-oldest old individuals (Table 3). Among the adults, 210 subjects (65,8%) were ISNA+, and 109 subjects (34,2%) were ISNA-. PR were the most frequent ISNA followed by Cs, MPS, and CLs categories. PR. Cs, and MPS were more common among the MCI types multiple domain than those single domain, while CLs didn't vary in the 4 MCI types. The mean number of ISNA was greater in the former than in the latter. Within the elderly, 405 subjects (88,4%) were ISNA+, and 53 subjects (11,6%) were ISNA-. The frequency of subjects ISNA+ didn't vary among the 4 MCI types. PR were the most frequent ISNA followed by Cs, MPS, and CLs categories. PR were almost equally distributed in the 4 MCI types, MPS and CLs were more common among the MCI types multiple domain, and Cs were more common among the nonamnestic MCI types. The mean number of ISNA was greater in the nonamnestic MCI types, than in the amnestic types. Among the old-oldest old subjects, 461 individuals (97,5%) presented at least one ISNA, and 12 individuals only (2,5%) were ISNA-. CLs and MPS didn't vary among the 4 MCI types. Cs were significantly more common among the nonamnestic MCI types, while PR were significantly more common among the amnestic MCI type. The mean number of ISNA was significantly greater in the na-MCImd type than in the other MCI types. The co-occurrence of multiple ISNA increased with age in all MCI types.

Baseline characteristics of MCI types ISNA+ and ISNA- are shown in Table 4. In all MCI types, no difference was found in the distribution of female and male among the two ISNA groups. In all MCI types, the mean age of female was greater in subjects ISNA+ than in subjects ISNA-, while the mean age of male was greater in subjects ISNA+ than in subjects ISNA- in the a-MCI only. Level of education didn't vary among the ISNA groups in MCI types single domain and in a-MCImd type, while in the na-MCImd type subjects ISNA+ were less educated than subjects ISNA-. The ADL scores were worse in the MCI types single domain ISNA+ only, and the IADL scores were worse in the MCI types single domain and a-MCImd ISNA+. In the na-MCImd type, ADL and IADL scores didn't vary among the two ISNA groups. Out of the VRF evaluated, arterial hypertension only was significantly more common among subjects ISNA+ than subjects ISNA- with the na-MCImd type, while no difference was found in the distribution of VRF among the two ISNA groups of the other MCI types. However, in a-MCI and na-MCImd, the VRF summary score was greater in subjects ISNA+ than those ISNA-. No difference was found in the frequency of the VD evaluated among subjects ISNA+ and ISNA- of almost all MCI types, with the exception of history of TIA that was significantly more common among the subjects a-MCI ISNA+. The VD summary score was greater in subjects ISNA+ than in those ISNA- in the a-MCI only.

In the a-MCI type, APOE ε4 carriers were significantly more common in subjects ISNA+ than in subjects ISNA-, and APOE ε4 non carriers were significantly more common in subjects ISNA- than in subjects ISNA+. The distribution of APOE ε4 carriers and non carriers didn't differ in the two ISNA groups of the other MCI types.

Neuropsychological performances of MCI types ISNA+ and ISNA- are summarized in Table 5. The MMSE score was above the cut-off level in all MCI types, but subjects a-MCI ISNA+ performed significantly less than subjects ISNA-. As expected, a worse performance on memory task was exhibited by the amnestic MCI types ISNA+ and ISNA-. In subjects with a-MCI, the nonmemory domains were not impaired, but subjects ISNA+ performed significantly less than subjects ISNA- in attention, executive function, and language. In subjects a-MCImd ISNA+ and ISNA-, the nonmemory domains were impaired, and subjects ISNA+ performed significantly worse than subjects ISNA- in tests evaluating language. In the nonamnestic MCI types no significant difference was found in the performance of nonmemory domains among subjects ISNA+ and ISNA-, although a trend of worse performance of subjects ISNA+ in almost all cognitive domains was evident.

Carotid ultrasonography and imaging findings in the MCI types ISNA+ and ISNA- are reported in Table 6. In all MCI types, the frequency of IMT was significantly higher in subjects ISNA+ than subjects ISNA-, while the frequency of SICA didn't differ between the two ISNA groups. In a-MCI, na-MCI, and na-MCImd, WMH-SC, WMH-T, and WMH-PV were significantly higher in subjects ISNA+ than subjects ISNA-, while in a-MCImd WMH-BG only were significantly higher in subjects ISNA+. Lacunes-BG and lacunes-T were significantly higher in MCI types single domain ISNA+, and lacunes-T were significantly higher in na-MCImd ISNA+. In a-MCI, na-MCI, and na-MCImd, SVD+ was more common in subjects ISNA+ than ISNA-, while SVD- was more common among the subjects ISNA- than ISNA+. In a-MCImd, the distribution of subjects SVD+ and SVD- didn't differ among the two ISNA groups. BCr was significantly higher in all MCI types ISNA+ with the exception of a-MCImd type. LVBr was significantly higher in the MCI types single domain ISNA+, while no difference among the ISNA groups was found in the MCI types multiple domains.

To assess the effects of the variables evaluated on the estimated probability of having at least one ISNA, logistic ridge regression analysis was carried out in each MCI type (Table 7). Age resulted associated with a-MCI type only. For each year of age increase, the odds of having at least one ISNA went up about 3%. Being female increased by 7,7% the probability of having at least one ISNA in the a-

MCI type only. Education, and VRF summary score didn't influence the probability of having at least one ISNA in any of the MCI types. VD summary score increased the probability of having at least one ISNA in a-MCI (18,1%), na-MCI (11,1%), and na-MCImd (4,5%). Being carrier of the APOE ε4 allele increased by 52,7%, 16,5%, and 5,1% the probability of having at least one ISNA in a-MCI, a-MCImd, and na-MCImd, respectively. At the increase of a single percentage point of the IMT the probability of having at least one ISNA in a-MCI, a-MCImd, na-MCI, and na-MCImd went up by 52,7%, 36,9%, 14,8%, and 16,3%, respectively. The presence of WMH-PV increased by 36,7%, 58,4%, and 14,3% the probability of having at least one ISNA in a-MCI, na-MCI, and na-MCImd, respectively. The presence of SVD in the frontal region increased the risk of having at least one ISNA in a-MCI (16,4%), na-MCI (54,5%), and na-MCImd (16%) types. The presence of SVD in the parieto-occipital region increased the risk of having at least one ISNA in a-MCI, na-MCI, and na-MCImd by 22,3%, 30,3%, and 10,5%, respectively. The presence of SVD in the lateral temporal region increased the risk of having at least one ISNA in a-MCI, a-MCImd, na-MCI, and na-MCImd MCI by 14,0%, 15,9%, 19,8%, and 8,4%, respectively. The presence of SVD in the basal ganglia increased the risk of having at least one ISNA in a-MCI, a-MCImd, na-MCI, and na-MCImd by 29,9%, 31,1%, 51,8%, and 9,7%, respectively. BCr, and LVBr resulted associated with all the MCI types. At the increment of a single percentage point of BCr, the probability of having at least one ISNA increased by 15,6% in a-MCI, 31,9% in a-MCImd, 65,8% in na-MCI, and 32,4% in na-MCImd. At the increment of a single percentage point of LVBr, the odds of having at least one ISNA increased by 13,1% in a-MCI, 6,2% in a-MCImd, 9,6% in na-MCI, and 5,2% in na-MCImd.

DISCUSSION

The frequency of ISNA detected in subjects with MCI was greater (86%) than that found in NCH aging individuals (57,2%).[20] In all MCI types, and in all age classes, PR were the ISNA most frequently encountered, followed by Cs, MPS, and CLs. All ISNA categories increased with age, and were presented by both the amnestic and nonamnestic MCI types, particularly by those multiple domain, with the exception of Cs and PR that in the old-oldest old subjects were more common among the MCI types single domain.

Copious data of the literature underline the importance of midlife vascular risks and Aβ burden to neurodegenerative processes, and to the development of cognitive decline in older adults. In CN

394 individuals, midlife VRF accelerate structural brain aging,[42] are associated with greater prospective
395 cognitive decline,[43] current and later-life smaller brain volumes,[44] and risk of dementia.[45] Further, midlife
396 but not later-life exposure to VRF is important also for Aβ deposition, as shown by the fact that having 2
397 or more midlife VRF compared with none is significantly associated with elevated Aβ deposition (61.2%
398 vs 30.8%).[46] In addition, in CN and MCI individuals, VRF interact with Aβ to reduce cortical thickness
399 in frontotemporal and parietal regions vulnerable to AD,[47] and are associated with prospective cognitive
400 decline, both alone and synergistically with Aβ burden.[48] However, VRF summary score was not
401 associated with ISNA in any of the 4 MCI types, and VD summary score increased the probability of
402 having at least one ISNA in the MCI types single domain, and poorly in the na-MCImd. These data need
403 attention. A high vascular risk burden at younger ages is indeed indicative of early vascular aging,[49] but in
404 the later years of life it is less relevant regarding the MCI typology since vascular and neurodegenerative
405 processes have already occurred at younger ages when the MCI typology is likely to be of single domain
406 type. The fact that subjects with MCI types multiple domain were older than subjects with MCI types
407 single domain supports this hypothesis. IMT increased the probability of having at least one ISNA in all
408 MCI types, and the risk was greater for the amnestic MCI types than for the nonamnestic types. These
409 results are in agreement with a previous study showing that IMT is associated with WMH, infarcts, brain
410 atrophy, and with poor cognitive performance particularly in the executive function.[50] Further, IMT
411 significantly increases the risk of conversion of a-MCI to dementia.[51] WMH-PV increased greatly the
412 probability of having at least one ISNA in MCI types single domain, and in the na-MCImd. It has been
413 shown that WMH-PV are associated with elevated Aβ deposition independently from age and presence of
414 APOE ε4 allele,[52] and induce thinning of prefrontal, parietal, temporal cortices, anterior insula, and
415 atrophy of the caudate nuclei,[14] and cognitive decline through the cortical atrophy of the above
416 disconnected regions.[19] Therefore, it is reasonable to suspect that even in our MCI types WMH-PV may
417 also have induced atrophy of the caudate nuclei, and thinning of the frontal, parietal, and temporal
418 cortices through likely disruption of periventricular long associating tracts.

419 Overall, SVD at different topographical locations, and BCr greatly increases the risk of having
420 ISNA in all the MCI types. Given that cortex, cerebellum, and BG are strictly interconnected,[53] it is
421 likely that ISNA are the by-product of the disconnection of the cortical-cerebellar-basal ganglia-thalamo-
422 cortical circuits induced by vascular and degenerative processes. It is also likely that the caudate atrophy
423 and the disruption of the internal circuits of BG may have induced an excessive inhibition to its output

nuclei. As a consequence, the inhibitory drive to thalamus may have led to bradykinesia, and that to brainstem structures controlling postural muscle tone and locomotion may have led to rigidity and gait/balance/axial dysfunction respectively,[54] while the inhibition of the physiological inhibitor control of lower brainstem centres on stereotyped motor responses[55] may have led to the reappearance of PR.

Nevertheless, the above hypotheses underpinning the vascular contribution to ISNA do not fully explain their presentation in the various MCI types. Among the amnestic MCI types, 39% (n=342) of subjects ISNA+ were SVD-, and 5% (n=45) of subjects ISNA- were SVD+. Similarly, among the nonamnestic MCI types 51% (n=190) of subjects ISNA+ were SVD-, and 35% (n=130) of subjects ISNA- were SVD+. These conflicting data suggest that co-occurring factors others than vascular contribute to the presentation of ISNA. The APOE ε4 allele can be one of these factors. In the present study, the APOE ε4 carriers were indeed over-represented relative to other studies, probably because the CogItA study assessed essentially subjects referred to the memory clinic, making the sample vulnerable to selection. Overall, APOE ε4 carriers were more common among subjects ISNA+ than subjects ISNA- in all MCI types, and being ε4 carrier increased the probability of having at least one ISNA greatly in the amnestic MCI types and scarcely in the nonamnestic MCI types, a finding in agreement with the notion that compared to non ε4 carriers, MCI ε4 carriers have frequently the amnestic phenotype.[56] The presence of APOE ε4 allele in nonamnestic MCI types is not surprising. Along with age, APOE ε4 allele is a significant predictor of amyloidosis. More than half of all MCI types is Aβ positive,[57] and APOE ε4 carriers are 2-3 times more likely to be amyloid positive than APOE ε4 non carriers.[6] Subjects with MCI have elevated Aβ deposition in frontal, parietal, temporal, and posterior cingulate cortices suggestive of early AD process,[58] and those converting to AD have a greater Aβ deposition in these regions, as well as in the putamen and in the caudate nuclei as compared to nonconverters.[59] Furthermore, in subjects with MCI the presence of the APOE ε4 allele is more frequent in the converters than in the nonconverters.[60] Therefore, it is reasonable to suspect that subjects of all 4 MCI types carrying the APOE ε4 allele were also Aβ positive, and that subjects ISNA+ being more APOE ε4 carriers than subjects ISNA- were candidates to convert to dementia faster than subjects ISNA-.

Limitations of the study

Some limitations of our study are worth noting. First, CSF biomarkers of Aβ and tau, and advanced imaging techniques up to now are not fully available in our country, particularly in a clinical

setting. So, we do not know the distribution of Aβ and tau in subjects ISNA+ and ISNA- of the various MCI types. Second, we assessed cortical and subcortical atrophy using linear measurements well aware that they are rather crude estimates of brain atrophy. However, it has been shown that the bicaudate ratio is a reliable marker of caudate atrophy,[61] and that ventricular enlargement is a feasible, even if nonspecific, surrogate marker of neurodegeneration in MCI and AD.[16] Third, we didn't estimate WMH volumetrically, but visually. However, it has been shown that WMH evaluated with visual scales correlate well with WMH volumetry.[62] Fourth, perhaps we underestimated the magnitude of cerebral SVD in our cohort, because we evaluated WMH and lacunes only. Fifth, the generalizability of our results is limited because the patients have been selected in a hospital setting. Sixth, in the present paper there is not mention of the ISNA in neurologically and cognitively normal subjects because data on this topic has been already published.[20] Lastly, the cross-sectional design of our study doesn't allow causal inferences.

CONCLUSION

ISNA are likely to have a multifactorial origin, increase with age, and are presented by both the amnestic and nonamnestic MCI types, particularly by those multiple domain, and carrying the APOE ε4 allele. Further, subjects ISNA+ perform less than subjects ISNA- in almost all nonmemory domains. Given that cortical and subcortical vascular and atrophic processes contribute to ISNA, their presence in individuals with MCI must alert the practitioners to target timely interventions to slowing cognitive decline and delay progression of MCI to dementia. Longer prospective population-based studies are needed to clarify to what extent the presentation of ISNA in middle-aged and older MCI individuals represents an additive risk for the conversion to dementia.

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STATEMENT OF AUTHORSHIP

CC and RC were responsible for the study's concept and design, data managements, and record linkage. GS and GC did the statistical analysis. CC, PT, CP, DA, and RC contributed to the analysis and interpretation of the data.

CC wrote the paper. All co-authors edited the paper, and approved its final version.

DISCLOSURES

All the authors hereby declare that they have nothing to disclose.

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Isolated, Subtle, Neurological Abnormalities in Mild Cognitive Impairment Types

Isolated, Subtle, Neurological Abnormalities in Mild Cognitive Impairment Types

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Abstract

Background: Isolated, subtle neurological abnormalities (ISNA) are commonly seen in aging, and have been related to cerebral Small Vessel Disease (SVD) and subcortical atrophy in neurologically and cognitively healthy aging subjects.

Objective: To investigate the frequency of ISNA in different MCI types, and to evaluate for each MCI type the cross-sectional relation between ISNA and white matter hyperintensities (WMH), lacunes, caudate atrophy, and ventricular enlargement.

Methods: 1,250 subjects with different MCI types were included in the analysis, and underwent brain MRI. WMH were assessed through two visual rating scales. Lacunes were also rated. Atrophy of the caudate nuclei and ventricular enlargement were assessed through the bicaudate ratio (BCr) and the lateral ventricles to brain ratio (LVBr), respectively. Apolipoprotein E (APOE) genotypes were also assessed. The routine neurological examination was used to evaluate ISNA that were clustered as central-based signs, cerebellar-based signs, and primitive reflexes. The items of Part-III of the Unified Parkinson's Disease Rating Scale were used to evaluate ISNA that were clustered as mild parkinsonian signs. Associations of ISNA with imaging findings was determined through logistic regression analysis.

***Results*:** The ISNA increase with the age, are present in all MCI types, particularly in those multiple domains, and carrying the APOE ε4 allele, and are associated with WMH, lacunes, BCr, and LVBr.

Conclusion: This study demonstrates that cortical and subcortical vascular and atrophic processes contribute to ISNA. Long prospective population-based studies are needed to disentangle the role of ISNA in the conversion from MCI to dementia.

Keywords: Isolated, subtle, neurological abnormalities, Mild cognitive impairment types, White matter hyperintensities, Lacunes, Caudate atrophy, Global cerebral atrophy

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INTRODUCTION

Mild cognitive impairment (MCI) is a term that refers to a condition in which an essentially spared global cognition, and normal or slightly impaired activities of daily living coexist with a mild decline of cognitive functions greater than that expected for age and education.[1,2] Four MCI phenotypes have been recognized[2] as follows: amnestic MCI single domain (a-MCI), amnestic MCI multiple domain (a-MCImd), nonamnestic MCI single domain (na-MCI), and nonamnestic MCI multiple domain (na-MCImd). Extracellular deposition of β-amyloid (Aβ) peptide, intracellular deposition of hyper-phosphorilated tau protein, and atrophy of frontal, parietal, and medial temporal cortices, *i.e.* neurodegeneration of AD signature cortical regions,[3] are the key elements of the pathophysiology of Alzheimer disease (AD),[4] and MCI due to AD.[5] The prevalence of Aβ positivity among subjects with MCI increases from age 50 to 90 years from 27% to 71%; nonamnestic MCI types have lower prevalence estimates of Aβ positivity than amnestic MCI types, but higher than subjects cognitively normal (CN), and both amnestic and nonamnestic MCI are at increased risk for AD.[6] Concerning tau, MCI and AD individuals have tau accumulation in the basal and mild-temporal, retrosplenial, posterior cingulate, and enthorinal regions greater than CN individuals Aβ positive.[7]

The observation that 21,5% and 35% of individuals with amnestic and nonamnestic MCI types, respectively, are Aβ negative,[8] has suggested that these individuals are not on the AD pathway, and that vascular pathology may be one of the possible non-AD causes of MCI. Among vascular pathology, cerebral small vessel disease (SVD) plays a pivotal role. SVD affects the smallest cerebral small vessels, increases throughout the lifespan,[9] and contributes to the risk of MCI,[10] and dementia.[11] White matter hyperintensities (WMH), lacunes, small subcortical infarcts, microbleeds, enlarged perivascular spaces, and central atrophy are the imaging markers of SVD.[12] Lacunes[13] and WMH[14] disrupting locally the structural integrity of white matter induce thinning of the connected cortical regions through Wallerian degeneration. Lacunes are associated with widespread cortical thinning, atrophy in multiple subcortical structures, and ventricular enlargement.[15] In subjects with MCI, expansion of the lateral ventricles is associated with atrophy of frontal, parietal, and temporal regions affected by AD.[16] Furthermore, age *per se* is associated with atrophy of the cerebellum, striatum, and prefrontal, parietal, and temporal association cortices.[17] The pattern of cortical atrophy induced by severe WMH overlaps substantially with the patterns of age-related cortical atrophy and of AD-related cortical atrophy.[18] Since cortical

atrophy induced by WMH drives cognitive decline,[19] age and SVD may contribute to the onset of cognitive decline through the overlapping atrophy of cortical regions vulnerable to AD pathology.

Cerebral SVD, Aβ, tau, and atrophic changes fragmenting over time brain networks into disconnected parts not only contribute to cognitive decline, but also contribute to the presentation of a wide range of neurological signs. We have shown that neurologically and cognitively healthy (NCH) aging subjects frequently present at the routine neurological examination isolated, subtle, neurological abnormalities (ISNA) which do not have any immediate diagnostic relevance, cannot be attributed to any definite, overt neurological disease, are associated with atrophy of the caudate nuclei, and with parietal WMH and lacunes, and probably constitute a red flag for future cognitive decline given that they show poor performance in test evaluating global cognition, executive function, and language.[20] Past reports on neurological signs in MCI have primarily focused on extrapyramidal features,[21,22] while reports on signs others than extrapyramidal are sparse. Therefore, the aims of our study are: 1) to investigate the prevalence of ISNA in the 4 MCI types; 2) to verify whether in the individual MCI types the probability of having ISNA is differently associated with the topographical location of WMH and lacunes, periventricular WMH (WMH-PV), APOE ε4 allele, and with two linear measures of central atrophy,[12] *i.e.* the bicaudate ratio (BCr) as proxy of subcortical atrophy, and the lateral ventricles to brain ratio (LVBr) as proxy of global brain atrophy. In the present paper, the terms "adult", "elderly", "old", and "oldest-old" will be used to indicate people aged 45-64, 65-74, 75-84, and >85 years respectively.

METHODS

Participants

Data were used from the Cognitive Impairment through Aging (CogItA) study, a hospital-based prospective study focused on normal and pathological aging in middle-aged and older individuals launched in January, 2000. CogItA's participants were outpatients self-referred or referred by general practitioners for neurological and/or cognitive screenings to the clinics of the Department of Neurology and Cognitive Disorders of the teaching Hospital (AOUP "P.Giaccone") of the School of Medicine of the University of Palermo, Italy. Details of the inclusion and exclusion criteria of the CogItA study have been reported elsewhere.[20] Informed consent was obtained from all participants and relatives. The study was approved by the University Hospital ethics committee, and complies with the declaration of Helsinki.

According to the published criteria,[1,2] CogItA participants with preserved global cognition at the Mini-Mental State examination (MMSE score ≥ 23,74),[23] subjective cognitive concerns, objective impairment in one or more cognitive domains, Clinical Dementia Rating (CDR) = 0.5),[24] no impaired or minimally impaired functional status on the activities of daily living (ADL)[25] and the instrumental activities of daily living (IADL) [26] scales, and no dementia were classified as MCI and categorized as a-MCI, a-MCImd, na-MCI, and na-MCImd. MCI subjects included in the present paper were stroke-free first ever diagnosed cases (n=1,250) aged 45-95 years (mean age = 70,52 ± 9,41 years), who remained in the MCI status for at least 3 years (mean follow-up = 64,98 ± 28,94 months). During this period, some of these subjects changed their MCI typology, but in the present paper first-ever MCI diagnoses were considered. Subjects who during the follow-up reverted to normal cognition, or converted to dementias different from AD and Vascular Dementia (VaD) were not considered.

Baseline Clinical Assessment

Participants to the present study underwent an extensive assessment of variables such as demographics, medical history, laboratory tests, neurological, and functional examinations, cognitive testing, carotid ultrasonography, and brain magnetic resonance imaging (MRI). Many vascular risk factors (VRF) and vascular diseases (VD) were considered and assessed as reported elsewhere.[20,29] Since multiple VRF and VD often coexist, we created the VRF and VD summary scores indicating for each participant the sum of the individual VRF and VD that were concurrently present. APOE genotypes were determined by using standard methods.[30] Participants with at least 1 APOE ε4 allele were classified as APOE ε4 carriers.

Assessment of ISNA

All participants underwent a standardised neurological examination reflecting that routinely performed in the clinical practice. Subjects presenting at baseline or during follow-up meaningful neurological signs such as visual field defects, language deficits, cranial nerves deficits, hemimotor and hemisensory dysfunction, brachial or crural weakness, brachial or crural sensory dysfunction, Babinski sign, spastic rigidity, and hemiplegic gait were excluded. The ISNA evaluated were: 1) mild dysphagia, 2) slurred speech, 3) central facial weakness, 4) mixed rigidity, *i.e.* a condition in which spastic and plastic rigidity coexist; 5) hyperreflexia (bilateral increased deep tendon reflexes), 6) reflex asymmetry, 7)

tremor (resting tremor, and postural/kinetic tremor), 8) plastic rigidity, 9) bradykinesia, 10) gait/balance/axial dysfunction, 11) dysmetria, 12) atactic type gait defined as a gait pattern broadly indicative of cerebellar involvement, and 13) primitive reflexes (PR) *i.e.* glabellar tap, snout, palmomental, grasping, and sucking reflexes.[31,32] To evaluate tremor, rigidity, bradykinesia, and gait/balance/axial dysfunction, collectively called Mild Parkinsonian Signs (MPS),[33] the items of motor section of the Unified Parkinson's Disease Rating Scale (UPDRS)[34] were used, and were considered present when any one of the following condition was met: (1) two or more items with a score of 1; or (2) one item with a score ≥ 2.[21] The ISNA were dichotomised as absent (score = 0) or present (score = 1), and were clustered into four categories as following: central-based signs (Cs) (signs 1-6), Mild Parkinsonian Signs (MPS) (signs 7-10), cerebellar-based signs (CLs) (signs 11-12), and PR (signs 13). We defined the presence of Cs, MPS, CLs, and PR as the presence of at least one sign within those included in each of these clusters. Accordingly, subjects were divided into subjects without ISNA (ISNA-), and with ISNA (ISNA+) if at least one sign within the above clusters was present. The neurological examination of each participant was always performed by two neurologists blinded to the patients history and neuroimaging. The interrater reliability assessed over time in random samples showed always excellent agreement with weighted Cohen's kappa ranging between 0.88 and 0.91 ($p< 0.001$).

Functional and Neuropsychological Assessments

The functional status of participants was assessed through the ADL and the IADL scales. Cognitive functions were assessed using an extensive neuropsychological battery as reported elsewhere,[20,35] including the MMSE as test of global cognition, and 12 tests to evaluate memory, attention, executive function, language, constructional ability, and visuospatial skill. Impaired cognitive domains were identified using a cut-off of 1,5 standard deviation (SD)[2] below the Italian normative data adjusted for age-, sex-, and education.[36]

Carotid Ultrasonography and Imaging Assessments

Intimal-medial thickness (IMT), and stenosis of internal carotid arteries (SICA) were assessed as reported elsewhere.[20] Participants had brain MRI on a 1,5T scanner (GE Signa HDxt, Milwaukee, WI, USA). Details of the image acquisition protocol have been published previously.[20] The BCr and the LVBr were calculated as reported elsewhere.[20,35] WMH, and lacunes were assessed according to the

published criteria.[12] The Wahlund scale (range 0-3) [37] was used to obtain the scores of WMH of the frontal, parieto-occipital, and temporal areas (WMH-SC), infratentorial (WMH-INF), basal ganglia (WMH-BG), and the WMH total score (WMH-T). To define the WMH status, a cut-off score ≥2 in at least one of the above regions was used. WMH-PV were evaluated with the Fazekas scale (range 0-3),[38] and a cut-off score ≥2 was used. Lacunes were assessed topographically according to the Wahlund regions used to score WMH, and categorized as lacunes-SC, lacunes-BG, lacunes-INF, and lacunes-T. A cut-off score ≥2 in at least one of the Wahlund regions was used to define the status of lacunes. Subjects having WMH and/or lacunes with a score ≥2 in at least one of the Wahlund scale topographical locations were categorized as SVD positive (SVD+), and those having WMH and/or lacunes with a score ≤1 were categorized as SVD negative (SVD-).

Statistical Analysis

Descriptive statistics (percentages, mean and SD, median and IQR) were used to summarise data. Continuous variables were compared between subjects ISNA- and ISNA+ by using one-way analysis of variance (ANOVA), and differences were tested with a post hoc F-test. Categorical variables were evaluated by contingency tables, and the hypotheses of independence were tested with $\chi 2$ test. Logistic ridge regression models[39] were used to evaluate for each MCI type the risk of having ISNA covarying for age, sex, years of education, and the variables found significant in the univariate analysis. In general, ridge regression method is the most applied solution for addressing problems of multicollinearity.[40] It implies adding a small positive constant (λ), *i.e.* the ridge parameter, to the main diagonal elements of the information matrix. The ridge parameter was selected using likelihood cross-validation.[41] IMT, BCr, and LVBr values were scaled to work on percentage of increments. All tests were two-tailed, and statistical significance was set at $p \leq 0.05$. Results are presented as odds ratios (ORs) with 95% confidence interval (95% CI). All analyses were performed using R statistical software (version 3.5.1; The R Foundation for Statistical Computing).

RESULTS

The demographic characteristics of the MCI types are shown in Table 1. In almost all MCI types, the majority of subjects were elderly followed by old, adult, and oldest-old individuals. The MCI types

single domain were more common among the adults, while those multiple domain were more common within the elderly, old, and oldest old participants. In each age class, the mean age didn't vary significantly among the various MCI types. Since the oldest old subjects were few, subsequent analysis was conducted pooling the old and oldest old classes in the class of old-oldest old.

Within the sample, 175 (14.0%) subjects didn't show any of the selected ISNA, while 1,075 (86.0%) presented at least one ISNA (Table 2). Overall, subjects ISNA- were more common among the MCI types single domain, and subjects ISNA+ were more common among those multiple domain. Primitive reflexes were the most frequent ISNA, followed by ISNA central-based, and mild parkinsonian signs, while ISNA cerebellar-based were the rarest. PR were exhibited by 864 subjects (69.1%) of the sample, and were more common among the MCI types multiple domain than those single domain. In all MCI types, snout reflex was the most common PR followed by glabellar tap, and palmomental reflex, while grasping and sucking reflexes were the rarest. Snout and palmomental reflexes were more common among the MCI types multiple domain than those single domain, while glabellar tap was more common among the amnestic MCI types than the nonamnestic types. The mean number of PR was greater among the amnestic MCI types than the nonamnestic types. Central-based signs were exhibited by 645 subjects (51,6%) of the sample. Subjects with Cs were more common among the nonamnestic MCI types than the amnestic types. In all MCI types, reflex asymmetry was the most common Cs, followed by bilateral hyperreflexia, central facial weakness, mixed rigidity, slurred speech, and dysphagia. Reflex asymmetry, bilateral hyperreflexia, and central facial weakness were more common among the nonamnestic MCI types than the amnestic types, while the frequency of the other Cs didn't vary among the 4 MCI types. The mean number of Cs was greater among the nonamnestic MCI types than the amnestic types. Mild Parkinsonian Signs were found in 46,2% (n=578) of the sample. In all MCI types, bradykinesia was the most common MPS, followed by gait/balance/axial dysfunction, and tremor, while rigidity was the rarest. Individual MPS were more common among the MCI types multiple domain than the MCI single domain. The mean number of MPS was greater in the former than in the latter. Cerebellar-based signs were the rarest ISNA encountered. Dysmetria was the most common CLs, and the atactic type gait was the rarest. The frequency of dysmetria didn't vary among the 4 MCI types, while the atactic type gait was more common among the a-MCImd than the other MCI types. The mean number of CLs didn't vary among the MCI types.

ISNA increased with age reaching a peak in the old-oldest old individuals (Table 3). Among the adults, 210 subjects (65,8%) were ISNA+, and 109 subjects (34,2%) were ISNA-. PR were the most frequent ISNA followed by Cs, MPS, and CLs categories. PR. Cs, and MPS were more common among the MCI types multiple domain than those single domain, while CLs didn't vary in the 4 MCI types. The mean number of ISNA was greater in the former than in the latter. Within the elderly, 405 subjects (88,4%) were ISNA+, and 53 subjects (11,6%) were ISNA-. The frequency of subjects ISNA+ didn't vary among the 4 MCI types. PR were the most frequent ISNA followed by Cs, MPS, and CLs categories. PR were almost equally distributed in the 4 MCI types, MPS and CLs were more common among the MCI types multiple domain, and Cs were more common among the nonamnestic MCI types. The mean number of ISNA was greater in the nonamnestic MCI types, than in the amnestic types. Among the old-oldest old subjects, 461 individuals (97,5%) presented at least one ISNA, and 12 individuals only (2,5%) were ISNA-. CLs and MPS didn't vary among the 4 MCI types. Cs were significantly more common among the nonamnestic MCI types, while PR were significantly more common among the amnestic MCI type. The mean number of ISNA was significantly greater in the na-MCImd type than in the other MCI types. The co-occurrence of multiple ISNA increased with age in all MCI types.

Baseline characteristics of MCI types ISNA+ and ISNA- are shown in Table 4. In all MCI types, no difference was found in the distribution of female and male among the two ISNA groups. In all MCI types, the mean age of female was greater in subjects ISNA+ than in subjects ISNA-, while the mean age of male was greater in subjects ISNA+ than in subjects ISNA- in the a-MCI only. Level of education didn't vary among the ISNA groups in MCI types single domain and in a-MCImd type, while in the na-MCImd type subjects ISNA+ were less educated than subjects ISNA-. The ADL scores were worse in the MCI types single domain ISNA+ only, and the IADL scores were worse in the MCI types single domain and a-MCImd ISNA+. In the na-MCImd type, ADL and IADL scores didn't vary among the two ISNA groups. Out of the VRF evaluated, arterial hypertension only was significantly more common among subjects ISNA+ than subjects ISNA- with the na-MCImd type, while no difference was found in the distribution of VRF among the two ISNA groups of the other MCI types. However, in a-MCI and na-MCImd, the VRF summary score was greater in subjects ISNA+ than those ISNA-. No difference was found in the frequency of the VD evaluated among subjects ISNA+ and ISNA- of almost all MCI types, with the exception of history of TIA that was significantly more common among the subjects a-MCI ISNA+. The VD summary score was greater in subjects ISNA+ than in those ISNA- in the a-MCI only.

In the a-MCI type, APOE ε4 carriers were significantly more common in subjects ISNA+ than in subjects ISNA-, and APOE ε4 non carriers were significantly more common in subjects ISNA- than in subjects ISNA+. The distribution of APOE ε4 carriers and non carriers didn't differ in the two ISNA groups of the other MCI types.

Neuropsychological performances of MCI types ISNA+ and ISNA- are summarized in Table 5. The MMSE score was above the cut-off level in all MCI types, but subjects a-MCI ISNA+ performed significantly less than subjects ISNA-. As expected, a worse performance on memory task was exhibited by the amnestic MCI types ISNA+ and ISNA-. In subjects with a-MCI, the nonmemory domains were not impaired, but subjects ISNA+ performed significantly less than subjects ISNA- in attention, executive function, and language. In subjects a-MCImd ISNA+ and ISNA-, the nonmemory domains were impaired, and subjects ISNA+ performed significantly worse than subjects ISNA- in tests evaluating language. In the nonamnestic MCI types no significant difference was found in the performance of nonmemory domains among subjects ISNA+ and ISNA-, although a trend of worse performance of subjects ISNA+ in almost all cognitive domains was evident.

Carotid ultrasonography and imaging findings in the MCI types ISNA+ and ISNA- are reported in Table 6. In all MCI types, the frequency of IMT was significantly higher in subjects ISNA+ than subjects ISNA-, while the frequency of SICA didn't differ between the two ISNA groups. In a-MCI, na-MCI, and na-MCImd, WMH-SC, WMH-T, and WMH-PV were significantly higher in subjects ISNA+ than subjects ISNA-, while in a-MCImd WMH-BG only were significantly higher in subjects ISNA+. Lacunes-BG and lacunes-T were significantly higher in MCI types single domain ISNA+, and lacunes-T were significantly higher in na-MCImd ISNA+. In a-MCI, na-MCI, and na-MCImd, SVD+ was more common in subjects ISNA+ than ISNA-, while SVD- was more common among the subjects ISNA- than ISNA+. In a-MCImd, the distribution of subjects SVD+ and SVD- didn't differ among the two ISNA groups. BCr was significantly higher in all MCI types ISNA+ with the exception of a-MCImd type. LVBr was significantly higher in the MCI types single domain ISNA+, while no difference among the ISNA groups was found in the MCI types multiple domains.

To assess the effects of the variables evaluated on the estimated probability of having at least one ISNA, logistic ridge regression analysis was carried out in each MCI type (Table 7). Age resulted associated with a-MCI type only. For each year of age increase, the odds of having at least one ISNA went up about 3%. Being female increased by 7,7% the probability of having at least one ISNA in the a-

MCI type only. Education, and VRF summary score didn't influence the probability of having at least one ISNA in any of the MCI types. VD summary score increased the probability of having at least one ISNA in a-MCI (18,1%), na-MCI (11,1%), and na-MCImd (4,5%). Being carrier of the APOE ε4 allele increased by 52,7%, 16,5%, and 5,1% the probability of having at least one ISNA in a-MCI, a-MCImd, and na-MCImd, respectively. At the increase of a single percentage point of the IMT the probability of having at least one ISNA in a-MCI, a-MCImd, na-MCI, and na-MCImd went up by 52,7%, 36,9%, 14,8%, and 16,3%, respectively. The presence of WMH-PV increased by 36,7%, 58,4%, and 14,3% the probability of having at least one ISNA in a-MCI, na-MCI, and na-MCImd, respectively. The presence of SVD in the frontal region increased the risk of having at least one ISNA in a-MCI (16,4%), na-MCI (54,5%), and na-MCImd (16%) types. The presence of SVD in the parieto-occipital region increased the risk of having at least one ISNA in a-MCI, na-MCI, and na-MCImd by 22,3%, 30,3%, and 10,5%, respectively. The presence of SVD in the lateral temporal region increased the risk of having at least one ISNA in a-MCI, a-MCImd, na-MCI, and na-MCImd MCI by 14,0%, 15,9%, 19,8%, and 8,4%, respectively. The presence of SVD in the basal ganglia increased the risk of having at least one ISNA in a-MCI, a-MCImd, na-MCI, and na-MCImd by 29,9%, 31,1%, 51,8%, and 9,7%, respectively. BCr, and LVBr resulted associated with all the MCI types. At the increment of a single percentage point of BCr, the probability of having at least one ISNA increased by 15,6% in a-MCI, 31,9% in a-MCImd, 65,8% in na-MCI, and 32,4% in na-MCImd. At the increment of a single percentage point of LVBr, the odds of having at least one ISNA increased by 13,1% in a-MCI, 6,2% in a-MCImd, 9,6% in na-MCI, and 5,2% in na-MCImd.

DISCUSSION

The frequency of ISNA detected in subjects with MCI was greater (86%) than that found in NCH aging individuals (57,2%).[20] In all MCI types, and in all age classes, PR were the ISNA most frequently encountered, followed by Cs, MPS, and CLs. All ISNA categories increased with age, and were presented by both the amnestic and nonamnestic MCI types, particularly by those multiple domain, with the exception of Cs and PR that in the old-oldest old subjects were more common among the MCI types single domain.

Copious data of the literature underline the importance of midlife vascular risks and Aβ burden to neurodegenerative processes, and to the development of cognitive decline in older adults. In CN

394 individuals, midlife VRF accelerate structural brain aging,[42] are associated with greater prospective
395 cognitive decline,[43] current and later-life smaller brain volumes,[44] and risk of dementia.[45] Further, midlife
396 but not later-life exposure to VRF is important also for Aβ deposition, as shown by the fact that having 2
397 or more midlife VRF compared with none is significantly associated with elevated Aβ deposition (61.2%
398 vs 30.8%).[46] In addition, in CN and MCI individuals, VRF interact with Aβ to reduce cortical thickness
399 in frontotemporal and parietal regions vulnerable to AD,[47] and are associated with prospective cognitive
400 decline, both alone and synergistically with Aβ burden.[48] However, VRF summary score was not
401 associated with ISNA in any of the 4 MCI types, and VD summary score increased the probability of
402 having at least one ISNA in the MCI types single domain, and poorly in the na-MCImd. These data need
403 attention. A high vascular risk burden at younger ages is indeed indicative of early vascular aging,[49] but in
404 the later years of life it is less relevant regarding the MCI typology since vascular and neurodegenerative
405 processes have already occurred at younger ages when the MCI typology is likely to be of single domain
406 type. The fact that subjects with MCI types multiple domain were older than subjects with MCI types
407 single domain supports this hypothesis. IMT increased the probability of having at least one ISNA in all
408 MCI types, and the risk was greater for the amnestic MCI types than for the nonamnestic types. These
409 results are in agreement with a previous study showing that IMT is associated with WMH, infarcts, brain
410 atrophy, and with poor cognitive performance particularly in the executive function.[50] Further, IMT
411 significantly increases the risk of conversion of a-MCI to dementia.[51] WMH-PV increased greatly the
412 probability of having at least one ISNA in MCI types single domain, and in the na-MCImd. It has been
413 shown that WMH-PV are associated with elevated Aβ deposition independently from age and presence of
414 APOE ε4 allele,[52] and induce thinning of prefrontal, parietal, temporal cortices, anterior insula, and
415 atrophy of the caudate nuclei,[14] and cognitive decline through the cortical atrophy of the above
416 disconnected regions.[19] Therefore, it is reasonable to suspect that even in our MCI types WMH-PV may
417 also have induced atrophy of the caudate nuclei, and thinning of the frontal, parietal, and temporal
418 cortices through likely disruption of periventricular long associating tracts.

419 Overall, SVD at different topographical locations, and BCr greatly increases the risk of having
420 ISNA in all the MCI types. Given that cortex, cerebellum, and BG are strictly interconnected,[53] it is
421 likely that ISNA are the by-product of the disconnection of the cortical-cerebellar-basal ganglia-thalamo-
422 cortical circuits induced by vascular and degenerative processes. It is also likely that the caudate atrophy
423 and the disruption of the internal circuits of BG may have induced an excessive inhibition to its output

nuclei. As a consequence, the inhibitory drive to thalamus may have led to bradykinesia, and that to brainstem structures controlling postural muscle tone and locomotion may have led to rigidity and gait/balance/axial dysfunction respectively,[54] while the inhibition of the physiological inhibitor control of lower brainstem centres on stereotyped motor responses[55] may have led to the reappearance of PR.

Nevertheless, the above hypotheses underpinning the vascular contribution to ISNA do not fully explain their presentation in the various MCI types. Among the amnestic MCI types, 39% (n=342) of subjects ISNA+ were SVD-, and 5% (n=45) of subjects ISNA- were SVD+. Similarly, among the nonamnestic MCI types 51% (n=190) of subjects ISNA+ were SVD-, and 35% (n=130) of subjects ISNA- were SVD+. These conflicting data suggest that co-occurring factors others than vascular contribute to the presentation of ISNA. The APOE ε4 allele can be one of these factors. In the present study, the APOE ε4 carriers were indeed over-represented relative to other studies, probably because the CogItA study assessed essentially subjects referred to the memory clinic, making the sample vulnerable to selection. Overall, APOE ε4 carriers were more common among subjects ISNA+ than subjects ISNA- in all MCI types, and being ε4 carrier increased the probability of having at least one ISNA greatly in the amnestic MCI types and scarcely in the nonamnestic MCI types, a finding in agreement with the notion that compared to non ε4 carriers, MCI ε4 carriers have frequently the amnestic phenotype.[56] The presence of APOE ε4 allele in nonamnestic MCI types is not surprising. Along with age, APOE ε4 allele is a significant predictor of amyloidosis. More than half of all MCI types is Aβ positive,[57] and APOE ε4 carriers are 2-3 times more likely to be amyloid positive than APOE ε4 non carriers.[6] Subjects with MCI have elevated Aβ deposition in frontal, parietal, temporal, and posterior cingulate cortices suggestive of early AD process,[58] and those converting to AD have a greater Aβ deposition in these regions, as well as in the putamen and in the caudate nuclei as compared to nonconverters.[59] Furthermore, in subjects with MCI the presence of the APOE ε4 allele is more frequent in the converters than in the nonconverters.[60] Therefore, it is reasonable to suspect that subjects of all 4 MCI types carrying the APOE ε4 allele were also Aβ positive, and that subjects ISNA+ being more APOE ε4 carriers than subjects ISNA- were candidates to convert to dementia faster than subjects ISNA-.

Limitations of the study

Some limitations of our study are worth noting. First, CSF biomarkers of Aβ and tau, and advanced imaging techniques up to now are not fully available in our country, particularly in a clinical

setting. So, we do not know the distribution of Aβ and tau in subjects ISNA+ and ISNA- of the various MCI types. Second, we assessed cortical and subcortical atrophy using linear measurements well aware that they are rather crude estimates of brain atrophy. However, it has been shown that the bicaudate ratio is a reliable marker of caudate atrophy,[61] and that ventricular enlargement is a feasible, even if nonspecific, surrogate marker of neurodegeneration in MCI and AD.[16] Third, we didn't estimate WMH volumetrically, but visually. However, it has been shown that WMH evaluated with visual scales correlate well with WMH volumetry.[62] Fourth, perhaps we underestimated the magnitude of cerebral SVD in our cohort, because we evaluated WMH and lacunes only. Fifth, the generalizability of our results is limited because the patients have been selected in a hospital setting. **Sixth, in the present paper there is not mention of the ISNA in neurologically and cognitively normal subjects because data on this topic has been already published.[20]** Lastly, the cross-sectional design of our study doesn't allow causal inferences.

CONCLUSION

ISNA are likely to have a multifactorial origin, increase with age, and are presented by both the amnestic and nonamnestic MCI types, particularly by those multiple domain, and carrying the APOE ε4 allele. Further, subjects ISNA+ perform less than subjects ISNA- in almost all nonmemory domains. Given that cortical and subcortical vascular and atrophic processes contribute to ISNA, their presence in individuals with MCI must alert the practitioners to target timely interventions to slowing cognitive decline and delay progression of MCI to dementia. Longer prospective population-based studies are needed to clarify to what extent the presentation of ISNA in middle-aged and older MCI individuals represents an additive risk for the conversion to dementia.

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STATEMENT OF AUTHORSHIP

CC and RC were responsible for the study's concept and design, data managements, and record linkage. GS and GC did the statistical analysis. CC, PT, CP, DA, and RC contributed to the analysis and interpretation of the data.

CC wrote the paper. All co-authors edited the paper, and approved its final version.

DISCLOSURES

All the authors hereby declare that they have nothing to disclose.

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