Factores genéticos y no-genéticos asociados a CADASIL: un estudio de cohorte retrospectivo

Nombre de la residente

Carolina Ospina Villegas

Programa

Neurología Clínica

Director de trabajo de investigación

Dr. Francisco Lopera Restrepo

Co-director de trabajo de investigación

Dr. Daniel Camilo Aguirre

Universidad de Antioquia

Facultad de Medicina

Programa de Neurología Clínica

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A mi mamá y a mi esposo,

por su confianza infinita para alcanzar esta meta

Contenido

1 Introducción

En los últimos treinta años, el Grupo de Neurociencias de Antioquia (GNA) ha caracterizado la presentación clínica y molecular de las demencias de inicio temprano en Antioquia, inicialmente mediante el estudio sistemático de la familia más grande que se conoce en el mundo con una sola mutación que causa la Enfermedad de Alzheimer de inicio temprano, y posteriormente de otras familias con diferentes enfermedades neurodegenerativas de origen genético, incluyendo CADASIL. Proyectos previos permitieron identificar 4 familias colombianas con esta última patología y caracterizarlas genotípica-fenotípicamente (1–8).

CADASIL representa un modelo puro de enfermedad cerebrovascular isquémica asociado a mutaciones en el gen NOTCH3 (9). Sin embargo, la presentación clínica y las edades de inicio varían de manera importante incluso en miembros de una misma familia (10–12), sugiriendo que existen otros factores subyacentes, que podrían modificar la evolución natural de la enfermedad.

CADASIL se caracteriza por una arteriopatía sistémica, que afecta específicamente a los pequeños vasos cerebrales (7). El daño se localiza sobre todo en las células del musculo liso de la capa media de los vasos, lo que contribuye a una disminución en la perfusión cerebral y la incapacidad de los vasos para la autorregulación, aumentando la susceptibilidad a la isquemia cerebral (7,13).

Por lo tanto, es posible plantear que diferentes factores ambientales que conlleven a exacerbar la arteriopatía subyacente o a incrementar la susceptibilidad tejido nervioso a las lesiones, podrían cambiar el curso clínico de la enfermedad, como los ya conocidos factores de riesgo cardiovascular.

El momento ideal para comenzar el tratamiento de cualquier enfermedad neurodegenerativa es antes de la aparición de los síntomas clínicos, permitiendo así que el paciente conserve sus funciones neurológicas durante el mayor tiempo posible. Si bien, actualmente no se puede modificar la carga genética de un individuo, si identificamos otros factores que puedan estar modificando la edad de inicio, tendríamos un impacto importante al retrasar la aparición de los ataques cerebrovasculares y con ello las secuelas físicas y cognoscitivas, tendríamos más años de vida productiva laboral y económica, con una menor carga social, emocional y económica en las familias y sus comunidades, al retrasar la discapacidad y la demencia, producto también de los múltiples ACV (14).

A continuación, se presenta el informe final con los resultados del proyecto de investigación realizado para optar al título de especialista en Neurología Clínica. Se presentan de acuerdo con los resultados esperados planteados en el proyecto de investigación.

La metodología a emplear será exponer lo realizado explicando cada objetivo asociado con las respectivas actividades realizadas para alcanzar el objetivo. Algunos objetivos tendrán asociados anexos que se detallan al final.

2 Objetivo general

Evaluar el papel de los factores de riesgo cardiovascular (hipertensión, diabetes, hipercolesterolemia, obesidad y tabaquismo) y las diferentes mutaciones en NOTCH-3 para explicar la variabilidad observada en la presentación clínica de la enfermedad.

3 Resultado específico 1

Dirigidos a la apropiación social del conocimiento

3.1 Actividades:

3.1.1 Reunión informativa a las familias de CADASIL sobre avances generales en la investigación: Se realizó una reunión en la SIU con pacientes y familiares para hacerlos partícipes de los avances de la investigación y fortalecer en ellos la educación en estilos de vida saludable.

3.1.2 Plegable informativo sobre CADASIL:
Se diseño un plegable informativo con la ayuda de neuropsicología y trabajo social sobre CADASIL, los síntomas asociados, su diagnóstico, forma de transmisión, entre otros. Estos se entregan durante las consultas, con el fin de llevar la información no solo al paciente, sino a los familiares que no pueden asistir. Ver Anexo 1.

4 Resultado específico 2

Otros aportes sociales del proyecto

4.1 Actividades:
Consultas médicas generales gratuitas:

Ante las dificultades de acceso a los servicios de salud, se prestó el servicio de consulta externa en medicina general a los pacientes y familiares con CADASIL. Se atendieron consultas de medicina general, con diagnósticos tales como: Hipertensión arterial esencial no controlada, EPOC descompensado, diabetes no controlada, insuficiencia venosa crónica, hipotiroidismo. Las historias clínicas, exámenes físicos y conductas quedaron debidamente registradas en SISNE.

5 Resultado específico 3

Productos resultados de actividades de investigación y divulgación de resultados a nivel nacional.

5.1 Actividades:

5.1.1 Poster titulado: “Relación entre el uso de ASA y la presencia de microhemorragias cerebrales en pacientes con CADASIL: resultados preliminares”. Presentado en el XIV Congreso Colombiano de Residentes, realizado del 16 al 18 de noviembre de 2018 en Yopal.

5.1.2 Poster titulado: “CADASIL como imitador en el diagnóstico de esclerosis múltiple”. Presentado en el XIII Congreso Colombiano de Neurología, realizado del 15 al 18 de marzo de 2018 en Barranquilla.

5.1.3 Presentación de trabajo en plataforma, como trabajo terminado. Factores genéticos y no-genéticos asociados a CADASIL: estudio de cohorte retrospectivo. En el XIV Congreso Colombiano de Neurología, que está programado para realizarse en noviembre de 2020 en Medellín

6 Resultado específico 4

Productos resultados de actividades de investigación y divulgación de resultados a nivel internacional

6.1 Actividades:

6.1.1 Poster titulado: “Genetic and nongenetic factors associated with CADASIL in Colombia: Preliminary results”. Presentado en el 14th Annual Canadian Neuroscience Meeting, realizado en Toronto en mayo de 2019.

6.1.2 Artículo titulado “Genetic and nongenetic factors associated with CADASIL: a retrospective cohort study” sometido a la revista Journal of the Neurological Science. Ver Anexo 2.

7 Resultado específico 5

Otros productos de formación o vinculación de la estudiante de especialidad en otros proyectos de investigación

7.1 Actividades:

7.1.1 Artículo terminado titulado "Cognitive performance in asymptomatic carriers of mutations R1031C and R141C in CADASIL". Yesica Zuluaga-Castaño, David Andrés Montoya-Arenas, Lina Velilla, Carolina Ospina, Joseph F. Arboleda-Velasquez, Yakeel T. Quiroz, Francisco Lopera. International Journal of Psychological Research. 2018; 11 (2): 46-55. Doi: 10.21500/20112084.3373. Ver Anexo 3.

7.1.2 Articulo terminado titulado: "The INECO Frontal Screening for the evaluation of executive dysfunction in cerebral small vessel disease: evidence from quantitative MRI in a CADASIL cohort from Colombia". Dorothee Schoemaker, Yesica Zuluaga, Anand Viswanathan, Markus Shrimer, Heirangi Torrico-Teave, Lina Velilla, Carolina Ospina, Gloria Garcia Ospina, Francisco Lopera, Joseph F. Arboleda-Velasquez and Yakeel T. Quiroz. Journal of the International Neuropsychological Society 2020; 3: 1–13. Doi: 10.1017/S1355617720000533. Ver Anexo 4.

7.1.3 Artículo en proceso de escritura, titulado "Specific abnormalities in white matter pathways as interface to small vessels disease and cognition in asymptomatic CADASIL patients".

8 Anexo 1

4 Cuál es el tratamiento?

Hasta el momento no existe tratamiento preventivo ni curativo.

35-55 años es la edad típica del diagnostico, sin embargo existe VARIABILIDAD en la edad de inicio, la velocidad de progresión y supervivencia entre pacientes.

Nuestras investigaciones han mostrado que un estilo de vida saludable, RETRASA el inicio de los síntomas.

5 Cómo puedes ayudarnos para avanzar juntos en la búsqueda de una solución?

- Participa en nuestros proyectos de investigación
- Deja conocer a tus familiares el deseo de donar tu cerebro para investigación en caso de que fallezcas.
- Participa en el voluntariado "No me olvides"

CADASIL

GNA

Sede de Investigación Universitaria SIU
Calle 62 No. 52 - 59
Teléfonos: 219 6424
Correo electrónico:
contacto@gna.org.co
www.gna.org.co

1 Qué es CADASIL?

Enfermedad poco frecuente.

Afecta las arterias pequeñas, causando múltiples infartos en el cerebro

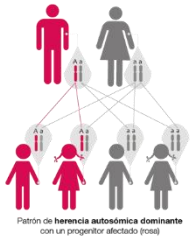
Gen NOTCH3

Es causada por mutaciones en el gen NOTCH 3, y por ello puede ser heredada.

Cada hijo tiene

50%

de probabilidades de heredarla, sin importar el sexo.



2 Cuáles son los síntomas?

Ataques cerebrovasculares RECURRENTES (cambios SÚBITOS en la fuerza, sensibilidad, habla, balance, coordinación, asimetría en la cara, perdida de la visión).

Deterioro **COGNITIVO** (repetición de ideas, olvidos de cosas recientes, desorientación en lugares conocidos) hasta la demencia

Trastornos **PSIQUIÁTRICOS** (cambios de personalidad, falta de interés por hacer cosas que disfrutaba, irritabilidad, desinhibición)

MIGRAÑA con aura.

3 Cómo saber si alguien tiene la enfermedad?

El diagnóstico lo debe hacer el médico, realizando una historia clínica de los síntomas del paciente y la historia familiar

Imágenes del cerebro como la resonancia ayudan a SOSPECHARLO por los cambios que quedan por los múltiples infartos.

El diagnostico definitivo es genético

9 Anexo 2

GENETIC AND NONGENETIC FACTORS ASSOCIATED WITH CADASIL: A RETROSPECTIVE COHORT STUDY

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AUTHORS: Carolina Ospina, MD.; Joseph F. Arboleda-Velasquez, M.D., Ph.D; Daniel Camilo Aguirre-Acevedo, PhD; Yesica Zuluaga-Castaño, MS; Lina Velilla, MS; Gloria Patricia Garcia, MS; Yakeel T. Quiroz, Ph.D; Francisco Lopera, M.D.

Carolina Ospina, MD, Neuroscience Group of Antioquia, University of Antioquia, Medellín, Colombia. Phone number 57-4-219 6424, carolina.ospinav@udea.edu.co

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CORRESPONDENCE SHOULD BE ADDRESSED TO:

Carolina Ospina, MD.

Neuroscience Group of Antioquia, Calle 62 #52–59, Lab 412, Torre 1, Sede de Investigación Universitaria, University of Antioquia. Medellín, Antioquia, Colombia. Email: carolina.ospinav@udea.edu.co Phone: 574- 219 6424 Fax: 574- 219 6444

ABSTRACT

Objective: To examine the relationships between genetic variables (genotype–phenotype) and cardiovascular risk factors in the natural history of CADASIL.

Methods: This was a retrospective cohort study of 331 individuals, 90 were carriers of four mutations in the NOTCH3 gene. Cox proportional hazards models were fitted to estimate the effect of genetic and cardiovascular factors on the onset of migraine, first stroke, and dementia. Competing risk regression models considered death as risk.

Results: Noncarriers and NOTCH3 mutation carriers had similar frequencies for all cardiovascular risk factors. Diabetes (SHR 3.5, 95% CI 1.75–7.15) was associated with a younger age at onset of strokes among carriers. Additionally, a genotype–phenotype relationship was observed among C455R mutation carriers, with higher frequency of migraines (100%), younger age at onset of migraine (median age 7 years, IQR 8) and cerebrovascular events (median age 30.5 years, IQR 26). Moreover, fewer carriers of the R141C mutation exhibited migraines (20%), and it was even lower than the frequency observed in the noncarrier group (44.8%).

Conclusions: This study characterizes extended family groups, allowing us a comparison in the genotype–phenotype. The results suggest a complex interplay of genetic and cardiovascular risk

factors, specially diabetes, that may help explain the variability in the clinical presentation and severity of CADASIL.

Keywords:

CADASIL, genotype, phenotype, migraine, diabetes, stroke

1. INTRODUCTION

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is the most common form of hereditary stroke disorder and typically presents with recurrent vascular episodes involving small vessels (1). It is often associated with migraines, neuropsychiatric disorders, cognitive deterioration, and dementia (2).

Although CADASIL is a single-gene disorder with mutations in the NOTCH3 gene, its clinical presentation can vary significantly (3–10), increasing the difficulty to suspect it and recognize a familial pattern, delaying diagnosis in new patients(11). Previous studies have reported variability in the age at clinical onset of the disease, with some individuals carrying the NOTCH3 mutations suffering their first stroke in their 20s and others in their 60s (3).

The prevalence of CADASIL has been estimated at 0.8 to 5 per 100,000 individuals (11–13) but under-diagnosis and misdiagnosis are suspected to be frequent in this entity (11,14).

Previous literature has reported inconsistent findings on the genotype-phenotype relationship, suggesting a role for other factors as modulators of the disease (9,11,15–17).

There is no specific disease-modifying treatment for CADASIL, and treatment focuses on the control of symptoms (18). Therefore, studying which factors can influence the age of onset of the main clinical characteristics of CADASIL (stroke, migraine, and dementia)? will allow future development and implementation of preventive interventions and inform prognosis.

The current study aimed to examine the role of genetic factors and cardiovascular risk factors (hypertension, diabetes, hypercholesterolemia, obesity, and smoking) in the variability observed in the clinical presentation and severity of the disease.

2. METHODS

2.1 Study design, setting, and participants.

This study is an observational retrospective cohort study. The descendants of individuals from families with NOTCH3 mutations were identified and included in the CADASIL Antioquia cohort study. It was conducted at the University of Antioquia in Colombia, South America, between January 1980 and December 2017 (10,17,19). Carriers and noncarriers of the NOTCH3 mutations R1031C, C445R, R141C, and R169C were included in this study. The initial database consisted of 514 individuals who were included in our Systematized Information System for the Neuroscience Group of Antioquia (SISNE) and were linked to the CADASIL project. However, for the purpose of this analysis, all participants who did not belong to one of the four families with CADASIL characterized in Antioquia (n = 115), those without a neurological medical evaluation in the Group of Neuroscience of Antioquia (n = 26), and those who were not properly genotyped for NOTCH3 mutations (n = 42) were excluded.

2.2 Standard protocol approvals, registrations, and patient consents.

Written informed consent was collected from all participants (or guardians of participants if the patients were too severely disabled) at the time of their evaluation. The study protocols and informed consent forms were approved by the medical ethics board of the University of Antioquia. The participants and the examiners were blinded to the genetic status of the individuals throughout the monitoring period.

2.3 Registers, clinical data, and definitions.

Demographic, clinical, and neuropsychological data of each patient were first collected with the help of a standardized form and then systematized with SISNE. The medical evaluation was carried out by neurologists or physicians who were trained in the evaluation of neurodegenerative diseases, whereas the neuropsychological evaluations were carried out by neuropsychologists or psychologists with training in the field of neuropsychology. All evaluations were carried out following a standardized protocol.

The data collected included age; gender; years of education; number of years repeating a grade; marital status; socioeconomic status; medical history; medication use; family history; Mini-Mental State Examination (MMSE) score in the last evaluation; behavioral changes; and presence of and age at the time of onset of stroke, migraines, memory complaints (referred by the patient or by a relative), and dementia.

In accordance with the Colombian legislation, the socioeconomic status was measured in levels ranging from 1 to 6, where levels 1–2 indicated “low income,” levels 3–4 were “middle income,” and levels 5–6 were “high income”(20). Behavioral changes were considered present if the medical records showed evidence of aggressiveness, jealousy, irritability, exhibitionism, isolation, apathy, disinhibition, or wandering.

Age at the time of onset of cerebrovascular disease was the patient's age at the time of first stroke, defined as an episode of acute neurological deficit lasting longer than 24 hours with no apparent cause other than of vascular origin (21). Age at the time of onset of dementia was defined in years by the neurologist who evaluated the patient, in accordance with the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (22). Migraine and migraine with aura were confirmed to be present if they fulfilled the definition provided in “The International Classification of Headache Disorders ICHD, 3rd edition (Beta version),” or by clinician criteria with the ICHD available at the time of evaluation, age at the time of onset was determined using the medical records (23).

Presence of and age at the time of onset of cardiovascular risk factors were also recorded. Hypertension was defined as being present if the patient or caregiver had reported a history of the disease, there was evidence of use of antihypertensive drugs, or the systolic blood pressure was greater than 140 mmHg or the diastolic blood pressure was greater than 90 mmHg in at least two evaluations, prior or concomitant to the age of the first stroke. Presence of diabetes mellitus was confirmed if the patient reported a history of the disease, showed evidence of using hypoglycemic medications, or exhibited altered laboratory results as per the criteria given by the American Diabetes Association, prior or concomitant to the age of the first stroke. Obesity was defined as a body mass index (BMI), calculated in kg/m2, greater than 30. Presence of hypercholesterolemia was confirmed if the total blood cholesterol was ≥200 mg/dL, or the patient reported a history of the disease with age of onset prior or concomitant to the age of the first stroke. Data on the history of smoking (current or former) included the number of years the patient had smoked, the age at which the patient began smoking, and the number of cigarettes the patient smoked per day. These data were self-reported by the patient or their companion.

2.4 Genetic analysis:

Genomic DNA was isolated from the peripheral blood using a PureGene DNA isolation kit (Gentra, Minneapolis, MN, USA). DNA from peripheral blood leukocytes of probands was amplified and each of the 33 exons including the intron–exon boundaries was sequenced. Relatives of the index cases were screened by gel-based length multiplex single base extension. Specific primers were used for the amplification of exons 8 and 19 of NOTCH3. The PCR products were evaluated using the following restriction enzymes: BsaHI (New England Biolabs) to identify the NOTCH3 mutation (R1031C) in exon 19 and the restriction enzymes BfmI (C455R), HgaI (R141C), and TauI (R169C) to detect the mentioned mutations in exon 8. The DNA fragments were visualized on 2% agarose gel, and the results were recorded under a numerical code to ensure blinding to genetic status.

2.5 Statistical analyses.

Categorical variables such as gender, socioeconomic status, marital status, hypertension, diabetes mellitus, obesity, hypercholesterolemia, smoking history, and migraine were presented as frequencies and percentages (%), whereas quantitative variables such as years of education, number of years repeating a grade, age of the first stroke, age at onset of dementia, migraine, migraine with aura, and MMSE were presented as mean and standard deviation or median and interquartile range (IQR), depending on the fulfillment of the assumption of normal distribution, evaluated using the Shapiro–Wilk test.

Genetic and environmental factors were compared between carriers and noncarriers using a chi-squared test for qualitative variables and a Fisher's exact test when the expected frequencies were less than 5. The quantitative variables were compared using a Mann–Whitney test. The differences were considered statistically significant if the p value was <0.05. Cerebrovascular disease-free survival curves for carriers and noncarriers were generated using the Kaplan–Meier method. Survival curves were also generated for the genotype groups and compared using a log-rank test or a Tarone–Ware test, depending on compliance with the assumption of proportional risks. The effect of cardiovascular risk factors (hypertension, diabetes mellitus, hypercholesterolemia, obesity, and smoking) on the onset of migraine, stroke, and dementia in the carrier group was assessed. Modified Fine and Gray competitive risks regression models were used to estimate the sub-hazard ratios (SHRs) and the corresponding 95% confidence intervals (CIs) (24,25). These models were generated after reclassification of the individuals; that is, participants who were alive and stroke free were considered as censored, whereas those who died without developing stroke were considered as competing risks, as death may preclude stroke occurrence or greatly alter the chances to observe it (25). Data was also corrected for age, sex and cardiovascular risk factors. All statistical analyses were performed using IBM SPSS 23.0, 2016 (IBM Corp., Armonk, NY, USA) and STATA 14 (StataCorp., College Station, TX, USA).

3. RESULTS

This study included 331 participants who had undergone clinical and genetic evaluation, with an average of 2.8 evaluations per person (IQR 4.1). The participants were followed up over time, with a maximum duration of 21 years in some patients. Of the 331 participants, 241 were noncarriers and 90 were carriers of mutations in NOTCH3. Among the carriers, 59 (65.6%) exhibited the R1031C mutation, 10 (11.1%) had the C455R mutation, 20 (22.2%) had the R141C mutation, and 1 (1.11%) exhibited the R169C mutation. Fifty-four (60%) carriers exhibited no symptoms of dementia or cerebrovascular events. The baseline characteristics of the carriers and noncarriers were quite similar (Table 1).

3.1 Clinical Characteristics of CADASIL

A history of migraine was seen in 39 (43.3%) carriers and 108 (44.8%) noncarriers. However, the differences in the frequencies of migraine and the age at the time of onset between the two groups

were not statistically significant (SHR = 0.96, 95% CI 0.65–1.41) (Figure 1). Among individuals with migraine, migraine with aura was the most common type in carriers (62.2%), and migraine without aura was observed more frequently in noncarriers (59.6%).

The mean score of MMSE at the last evaluation did not significantly differ between carriers (median = 28, IQR = 6) and noncarriers (median = 29, IQR = 3). Moreover, not statistically significant between-group differences were seen in the age at the time of onset of memory complaints (carriers: median = 44 years, IQR = 14; noncarriers: median = 40 years, IQR = 20.8). In contrast, behavioral changes were observed more frequently in the carrier group (33.3% vs. 11.2%; $p < 0.001$).

The survival curves for age at the time of onset of dementia have been shown in Figure 1. At the end of the follow-up period, only 3 (1.2%) noncarriers and 14 (15.5%) carriers met criteria for a clinical diagnosis of dementia. The age at the time of onset of dementia was earlier in NOTCH3 mutation carriers, with a median value of 65 years.

A positive history of stroke was observed in 33 (36.6%) carriers and 5 (2.1%) noncarriers. None of the noncarriers had experienced three or more cerebrovascular events, whereas 25% of those with cerebrovascular disease in the carrier group had done so. The median age at the time of the first cerebrovascular event in the carriers was 56 years (95% CI 46–69). The age at the time of onset of cerebrovascular events was earlier in NOTCH3 mutation carriers compared with the noncarriers (SHR = 20.6, 95% CI 8.4–50.4). The survival curves have been shown in Figure 1.

3.2 Cardiovascular risk factors in CADASIL

The number of individuals presenting with cardiovascular risk factors were similar between the carrier and noncarrier groups (Table 2). After adjusting for death as a competing risk factor, diabetes (SHR 3.5, 95% CI 1.75–7.15) was seen to be associated with an earlier age at the time of onset of stroke. No association between age at the time of onset of stroke and hypertension (SHR 0.93, CI 95% 0.41–2.10), hypercholesterolemia (SHR 0.90, 95% CI 0.33–2.46), smoking (SHR 2.08, CI 95% 0.95–4.58), or obesity (SHR 0.48, CI 95% 0.05–4.41) (Figure 2) was observed.

3.3 Phenotype and genotype correlation analysis

R169C carriers were not included in the phenotype and genotype analysis because of the limited number of cases (n = 1). This individual experienced migraine with aura at the age of 12, the first cerebrovascular event at the age of 36 years and reported no memory complaints or dementia until the time of his last evaluation. Moreover, this individual also did not exhibit any of the cardiovascular risk factors described previously.

The C455R, R1031C, and R141C NOTCH 3 mutation groups exhibited similar demographic characteristics, with no significant differences in the distribution by sex, marital status, years of schooling, the number of years repeating a grade, age at the time of first evaluation, age at the time of last medical evaluation and age at the time of death being observed (Table 3).

The C455R carriers exhibited an earlier age of onset of migraine (median age 7 years, IQR 8, $p = 0.001$), whereas the corresponding ages in the R1031C carrier (median age 18 years, IQR 14.5), R141C carrier (median 19 years, IQR 13.3), and the noncarrier (median 15 years, IQR 10.5) groups were largely similar (Figure 3). These findings were maintained when comparing only individuals with migraine with aura. A greater proportion of C455R carriers exhibited migraines (n = 10, 100%) compared with the noncarriers (n = 108, 44.8%), the R1031C carriers (n = 24, 40.7%), and the R141C carriers (n = 4, 20%). The number of individuals with the R141C mutation that presented with migraine was even lower than the frequency observed in the noncarrier group (Figure 4).

Statistically significant differences in the age at the time of onset of cerebrovascular disease were observed, with C455R carriers exhibiting an earlier age of onset (median age 30.5 years, IQR 26), followed by the R141C carriers, and then the R1031C carriers (log-rank $p < 0.001$) (Figure 3). A greater number of C455R carriers exhibited the presence of diabetes mellitus 3 (30%, $p = 0.033$), hypercholesterolemia 6 (60%, $p < 0.001$), and smoking 8 (80%, $p = 0.003$), although the age at the time of onset of these factors did not differ significantly from that observed among the noncarriers and R1031C and R141C mutation carriers. No differences in age at the time of onset of dementia were observed between the different NOTCH3 mutations (Figure 3).

4. DISCUSSION

Initial case reports defined CADASIL as a hereditary multi-infarct dementia that occurred in non-hypertensive patients (26), and the clinical diagnostic criteria, which are used even today, included "the absence of risk factors for cerebrovascular events." (27,28). However, the results of the current study showed that a diagnosis of CADASIL frequently coexisted in patients with a history of cardiovascular risk factors, with a similar frequency to that seen in noncarriers who share similar environments, including hypertension. These results were consistent with previous reports (9,11,15,16), thus highlighting the need for reassessment of the clinical criteria and increased awareness in daily practice.

Although the relationship between the cardiovascular risk factors and the development of stroke and vascular dementia has been described previously (29), how these conditions modify the clinical features of hereditary cerebrovascular diseases such as CADASIL is still unclear. A limited number of studies have reported an association between smoking and age at the time of onset of lacunar infarcts, incidence of stroke, and dementia (9,30,31).

The COX regression analysis carried out in this study allowed estimation of the effect of environmental and genetic factors on the age at the time of onset of stroke. The results showed a statistically significant association between diabetes and age at the time of onset of cerebrovascular disease. An association between the levels of glycosylated hemoglobin and the number of brain microbleeds in CADASIL has been reported previously, but not with clinical stroke and its age of onset (32). Other studies have highlighted the deleterious role of smoking and hypertension (9,31), however, this is the first study to report a correlation between the early onset of ischemic cerebrovascular events and diabetes. Therefore, a more aggressive approach to diabetes in its prevention and treatment may delay the onset of cerebrovascular events in NOTCH3 mutations carriers.

CADASIL is the most frequent cause of hereditary cerebrovascular disease (1,33,34). However, despite being a single-gene disorder, it exhibits large phenotypic variability, resulting in great inconsistency in the results of studies that have tried to demonstrate genotype–phenotype relationships (3,7–10,30,31,35,36).

The findings of the current study suggest the existence of a genotype–phenotype relationship in CADASIL patients, with R1031C mutation carriers exhibiting later onset of cerebrovascular events and C455R mutation carriers being the youngest at the time of onset of such events. C455R mutation is located at the ligand-binding domain of Notch3 and its phenotypic characteristics suggests increased severity of the phenotype. Our findings are not consistent with other recent research, where CADASIL patients with an EGFr 1–6 pathogenic variants were associated with a more severe CADASIL phenotype (37). Since C455R mutation showed the earlier onset of stroke and is located in the EGFr domain 11, additionally even though R141 mutation is located in the EGFr domain 3, it did not statistically differ from R1031C mutation located in the EGFr 26 in terms of frequency of migraine, stroke, dementia or ages of onset.

Moreover, the frequency and age at the time of onset of migraine appear to be associated with the type of mutation present, with a higher frequency of migraine and an earlier age at the time of onset in C455R carriers. A smaller number of individuals with the R141C mutation exhibited migraines compared with the other mutations and the noncarrier groups (Figure 3). Similar results have been reported among other patients with the same mutation in Japan (38). These results lead us to question whether we are currently facing a clinical CADASIL spectrum that does not include migraines, or if the association between CADASIL and migraines is genotype dependent.

This study has several limitations. Given that it was a retrospective analysis, there were several methodological difficulties and potential biases. Over more than 35 years of medical evaluations, data was collected by people with different medical training, some with specialized training, and inter-evaluator reliability and reproducibility are not clear for some definitions as dementia and behavioral changes. Age of onset of hypertension, diabetes and hypercholesterolemia was defined as the age of diagnosis, but this should be considered a limitation since it is not the beginning of the disease or the physiological alteration they cause. The sample size was relatively small, and the CIs were wide. Therefore, negative findings, such as the association between smoking and age at the time of onset for cerebrovascular disease (9,31), should be interpreted with caution. A larger sample size would result in greater statistical strength of the findings. Dementia cases were relatively low, compared to previous studies with reported frequencies of dementia between 25-28% (7). In CADASIL mean age for dementia diagnosis is 58.2 years (8), but our population is relatively young, the median age of the last evaluation was 42 years in carriers, possibly due to the active search for carriers even in asymptomatic stages. It is necessary to continue their clinical follow up over time.

However, this study also has several important strengths. An important strength of the study is the long follow-up period and the fact that many participants did not suffer from clinical cerebrovascular disease or dementia at the beginning. To the best of our knowledge, there are no other studies that have described the clinical evolution of both symptomatic and asymptomatic patients with NOTCH3 mutations over time, nor taken such large family groups into consideration. Second, comparison with noncarriers belonging to the same family groups permitted examination of individuals exposed to similar environmental and cultural factors. Third, inclusion of the siblings of index cases allowed us to characterize extended family groups, which facilitated a more robust comparison in the genotype–phenotype analysis.

Overall, the results of this study showed that the frequency of cardiovascular risk factors was similar between carriers of NOTCH3 mutations and noncarriers. Additionally, CADASIL was characterized by great phenotypic variability that, in our study, was influenced by genetic and nongenetic factors. The findings also indicate that the presence of diabetes may accelerate the onset of cerebrovascular events in NOTCH3 mutation carriers.

5. ACKNOWLEDGMENT:

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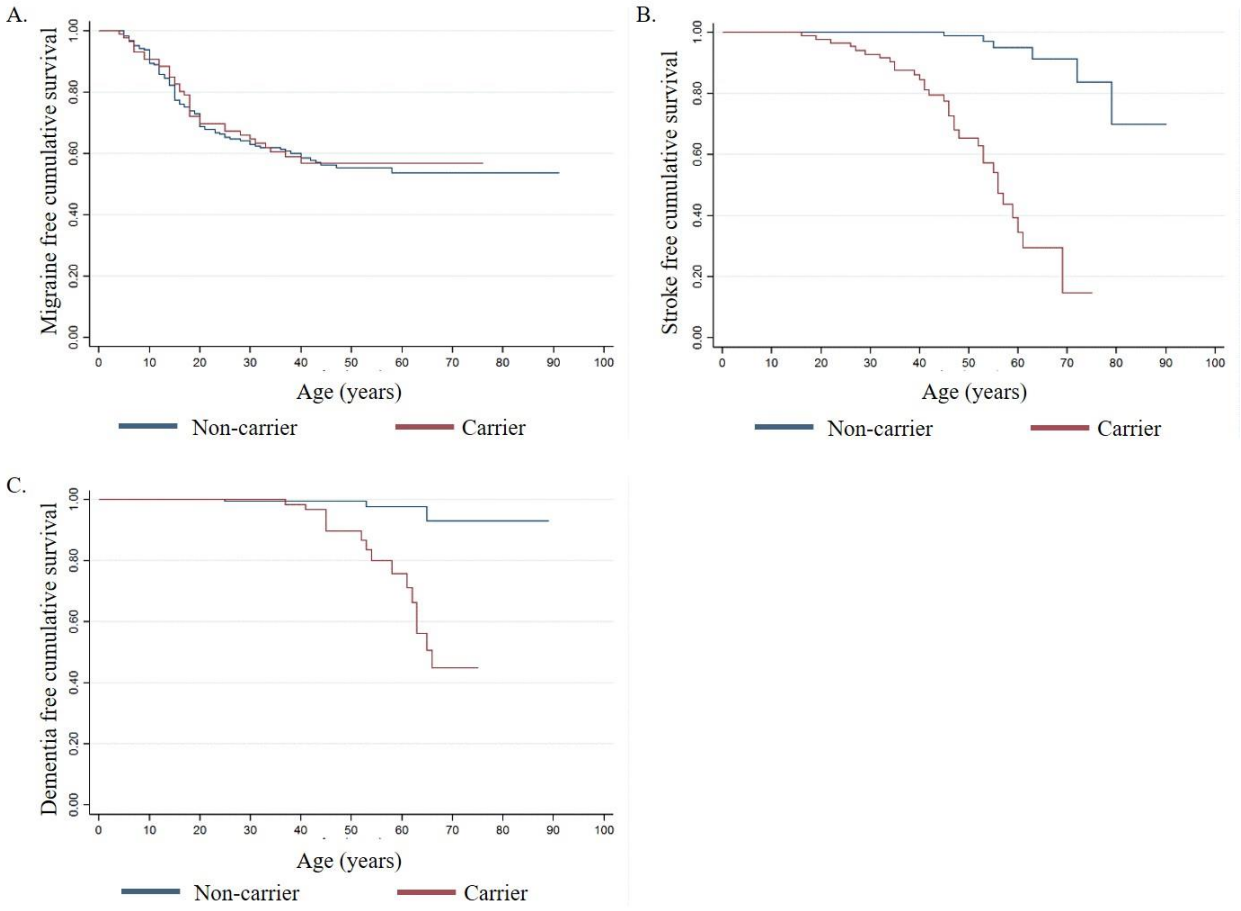
7. TABLES & FIGURES:

Table 1. Baseline characteristics of carriers and noncarriers of NOTCH3 mutations

	Noncarriers n = 241	**Carriers n = 90**	**p value**
Female, n (%)	158 (65.6)	49 (54.4)	0.063
Socioeconomic status, n (%)			0.002
Low income	83 (56.8)	18 (42,9)	
Middle income	59 (43.2)	9 (57,1)	
High income	0 (0)	0 (0)	
Marital status, n (%)			0.903
Single	83 (35)	31 (34.8)	
Married	94 (39.7)	37 (41.6)	
Widower	14 (5.9)	3 (3.4)	
Free union	33 (13.9)	12 (13.5)	
Divorced	13 (5.5)	6 (6.7)	
Years of education, median (IQR)	5 (8)	5 (11)	0.490
Number of years repeating a grade, median (IQR)	0 (1)	0 (1)	0.604
Age at first evaluation, median (IQR)	36 (24.0)	35.5 (21)	0.713
Age at final evaluation, median (IQR)	41 (26)	42 (20.3)	0.085
Age at death, median (IQR)	86.5 (47.5)	57.5 (19.3)	0.108

Abbreviations: IQR = interquartile range

Figure 1. Migraine, Stroke and dementia free cumulative survival analysis in NOTCH3 carriers vs noncarriers

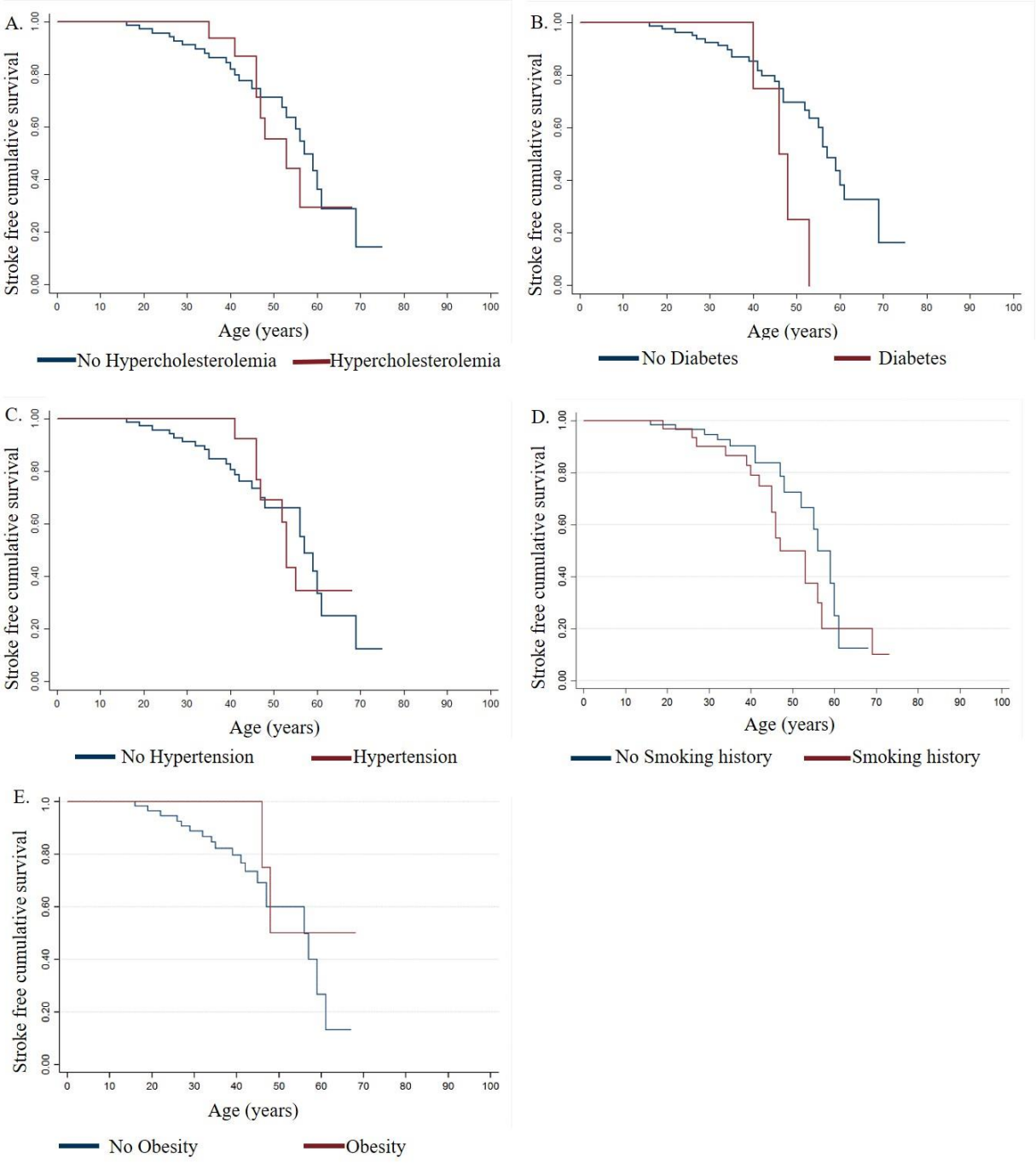


Kaplan-Meier plot. A. Migraine free cumulative survival, B. Stroke free cumulative survival, C. Dementia free cumulative survival.

Table 2. Cardiovascular risk factors profile in carriers and noncarriers of NOTCH3 mutations

	Noncarriers n = 241	**Carriers n = 90**	**p Value**
Hypertension, n (%)	44 (18.3)	15 (16.6)	0.737
Diabetes Mellitus, n (%)	13 (5.4)	5 (5.6)	0.954
Obesity, n (%)	8 (3.3)	6 (6.7)	0.249
Hypercholesterolemia, n (%)	26 (10.8)	14 (15.6)	0.236
Smoking, n (%)	60 (24.9)	30 (33.3)	0.125

Figure 2. Stroke free cumulative survival analysis in NOTCH3 mutation carriers



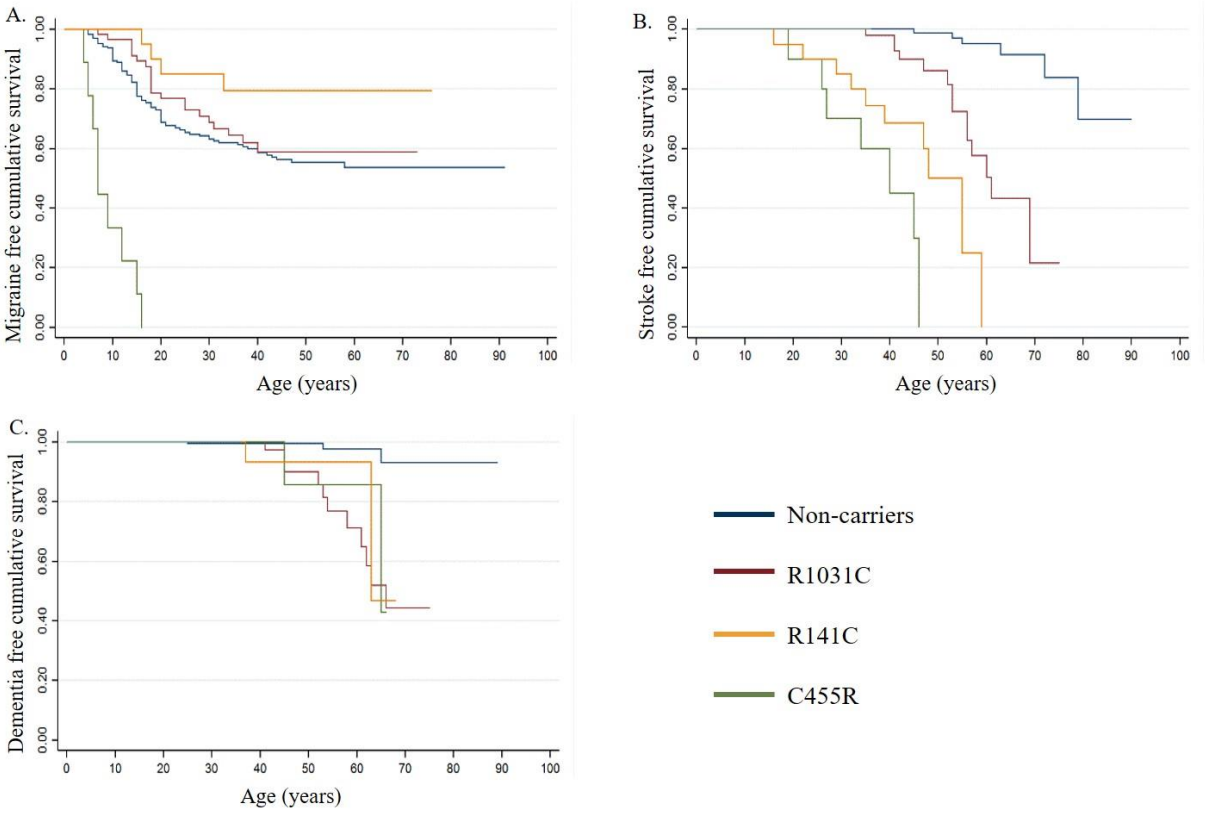
Kaplan-Meier plot A. Hypercholesterolemia B. Diabetes Mellitus C. Hypertension D. Smoking Hypertension E. Obesity

Table 3. Baseline characteristics of carriers and noncarriers of NOTCH3 mutations

	Control n = 241	**R1031C n = 59**	**C455R n = 10**	**R141C n = 20**	**Valor p**
Female, n (%)	158 (65.6)	31 (52.5)	6 (60.0)	12 (60.0)	0.267
Socioeconomic status, n (%)					0,000
Low income	83 (56.8)	14 (41.2)	1 (11.1)	12 (63.1)	
Middle income	63 (43.2)	20 (58.8)	8 (88.9)	7 (36.8)	
High income	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Marital status, n (%)					0.822
Single	83 (35.0)	21 (36.2)	4 (40.0)	6 (30.0)	
Married	94 (39.7)	23 (39.7)	2 (20.0)	11 (55.0)	
Widower	14 (5.9)	2 (3.4)	1 (10.0)	0 (0.0)	
Free union	33 (13.9)	8 (13.8)	1 (10.0)	3 (15.0)	
Divorced	13 (5.5)	4 (6.9)	2 (20.0)	0 (0.0)	
Years of education, median (IQR)	5 (8.0)	5 (9.0)	8.5 (5.5)	5 (8.0)	0.579
Number of years repeating a grade, median (IQR)	0 (1.0)	0 (1.0)	1 (2.0)	0.5 (2.0)	0.065
Age at first evaluation, median (IQR)	36 (24.0)	33 (21.0)	39 (23.3)	38.5 (14.8)	0.487
Age at final evaluation, median (IQR)	41 (26.0)	42 (24.0)	50 (24.5)	42 (17.0)	0.175
Age at death, median (IQR)	86.5 (47.5)	58 (17.0)	51 (30.8)	73 (0.0)	0.206

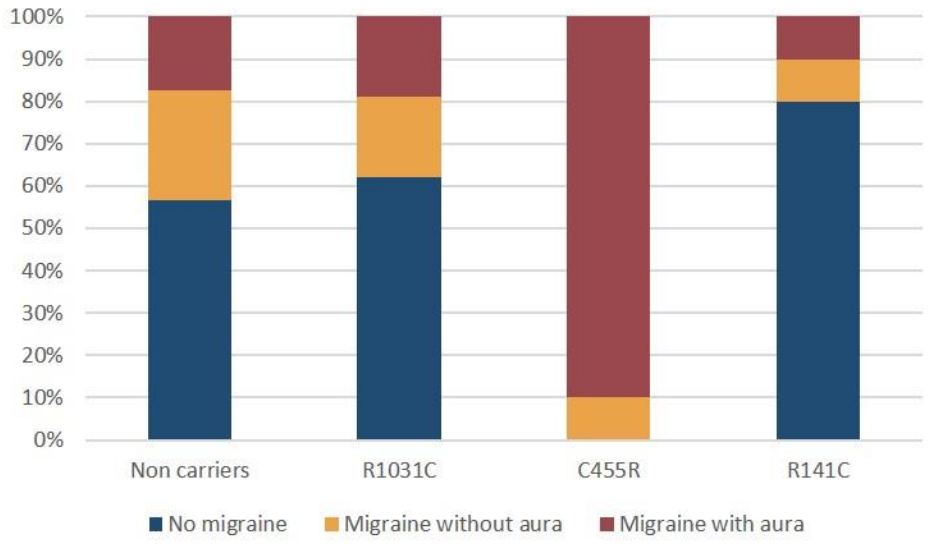
Abbreviations: IQR = interquartile range

Figure 3. Time to event analysis in different NOTCH 3 mutations



Kaplan-Meier plot. A. Migraine free cumulative survival, B. Stroke free cumulative survival, C. Dementia free cumulative survival.

Figure 4. Migraine in carriers and noncarriers of NOTCH3 mutations



8. APPENDIX

This study is an observational retrospective cohort study. This design has the advantage that it allows studying rare diseases as CADASIL, analyzing pre-existing data, which was obtained during many years of clinical monitoring.

However, due to its retrospective nature, it has a significant risk of bias and confounding factors, which are necessary to consider, and how attempts were made to avoid them is discussed here.

- Selection of subjects: A critical characteristic of subject selection is to select both groups from the same source population (39). In the study, examiners must remain blind to the genetic status of individuals, therefore data collection was done in the entire population of CADASIL patients and their relatives available in SISNE. Only after data collection from medical records was done, individuals were assigned with a numerical code and the person in charge of the genetic studies provided a new database with the status of carrier and non-carrier for the statistical analysis.

- Recall Bias: data on presence and age at the time of onset of stroke, migraine, hypertension, diabetes, smoking was collected by medical interviews. Most of the time, individuals attended the medical evaluation with a caregiver or companion who can help in the process of remembering. Examiners had available standardized medical forms to use during medical evaluation through the Systematized Information System for the Neuroscience Group of Antioquia (SISNE).

- Information bias: standardized medical forms were not specifically designed for the study, and fulfillment of every question in the forms was not mandatory for examiners. As we worked on a retrospective database, it was not possible for us to guarantee the quality of the data and we had to work on those that were already registered. This limited the number of variables that we could analyze and that could also have an impact on the age of onset of stroke in these patients, such as number of cigarettes per year, years of smoking, physical activity, the effect of adequate or inadequate control of hypertension, diabetes or hypercholesterolemia over years and insulin use requirements.

- Interviewer Bias: Data was collected by examiners previous to the design of this study. And to avoid this bias in how the reviewer searches for information on medical records more diligently for one group, he was blinded from the genetic status and performed these searches through all medical records (CADASIL patients and relatives).

- Temporal relationships: The age of onset of the first stroke and the age of onset of hypertension, diabetes, hypercholesterolemia was extracted from the medical records. The cardiovascular risk factor was defined as present, only if the registered age of onset was prior or concomitant to the age of the first stroke.

- Age of onset of hypertension, diabetes, and hypercholesterolemia was defined as the age of diagnosis, but this should be considered a limitation since it is not the beginning of the disease or the physiological alteration they cause.

- Misclassification bias: an electronic standardized data collection instrument was used. The variables collected were defined and determined a priori and documented in a coding guide for reviewers. But variables definitions have important limitations. Data was collected since 1980, and the definitions of stroke, diabetes, hypertension have changed over time. For this reason, it has been necessary to use broad definitions, which would allow clinical diagnosis even in those times. For example, stroke was defined using the World Health Organization definition, which did not require confirmation by neuroimaging, but was guided by clinical criteria (21).

We are aware of the limitations of the study design, we tried to avoid some of its possible bias, but it was not always possible. Even though our findings may be used as the initial study generating hypotheses to be studied further by larger prospective studies.

10Anexo 3

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11Anexo 4

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