The Egyptian Journal of Neurology, Psychiatry and Neurosurgery

RESEARCH

Open Access

Whole exome sequencing in a sample of Egyptian patients with covert cerebral small vessel disease

Hany Aref[1], Mohamed Maged[1*], Tamer Roushdy[1], Hossam Shokri[1], Eman Hamid[1], Bernard P. H. Cho[2], Hugh S. Markus[2], Mai Fathy[1] and Nevine El Nahas[1]

Abstract

Background Covert cerebral small vessel disease (cCSVD) is associated with many age-related morbidities with little available data regarding the pathophysiology and role of genetics in it. This study aims to investigate the genetic load in a sample of Egyptian patients with cCSVD.

Results Thirty patients with cCSVD were recruited and underwent cognitive, gait, sphincter assessment, magnetic resonance imaging (MRI) brain, and blood sampling for whole exome sequencing. The mean age for the patients was 65.93 ± 8.8 with male patients representing 63.33% of the studied sample. The major risk factor was hypertension followed by diabetes mellitus, dyslipidaemia, and smoking. The main presenting symptom was cognitive impairment, found in 60% of the patients and the mean duration of symptoms was 2.1 ± 1.12 years. Two out of thirty patients were positive for a known pathogenic gene (NOTCH3 and COL4A1) despite the absence of family history in one representing 6.7% of the entire studied sample. Meanwhile, three patients had variant genes not previously linked to cCSVD.

Conclusions Whole exome sequencing and genetic studying of patients with cCSVD is of utmost importance as the genetic load is underestimated in the Egyptian population.

Keywords Cerebral small vessel disease, Monogenic, Whole exome sequencing, Vascular dementia

Introduction

Cerebral small vessel disease (CSVD) refers to various pathological processes affecting the brain's small arteries, arterioles, capillaries, and venules [1].

To date, a monogenic cause has been identified only in a minority of families with clustering of stroke. In clinical practice, routine genetic testing is commonly performed for a minority of well-defined pathologies, such as cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). It is, therefore, likely that many other gene variations remain undiagnosed. This can result in an under-representation of the contribution of these monogenic forms to the overall genetic stroke risk [2].

In Africa and the Arab world, even fewer studies and reports of a monogenic cause of CSVD exist, with the main focus on CADASIL as previously three families in Saudi Arabia, Kuwait, and Yemen were studied and discovered to have a total of 19 individuals with NOTCH3 mutation [3]. Another report was of a Gabonese man with CSVD who was diagnosed with cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) having heterozygous HTRA1 mutation [4].

*Correspondence:
Mohamed Maged
m_maged197@hotmail.com
[1] Neurology Department, Faculty of Medicine, Ain Shams University, 38 Abbasia, Cairo 11591, Egypt
[2] Stroke Research Group, Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK

This study aims to assess the genetic load and monogenic causes in a sample of Egyptian patients with covert cerebral small vessel disease (cCSVD).

Methods

This is a cross-sectional descriptive study. It aims to assess 30 patients with cCSVD and measure the prevalence of monogenic causes using whole exome sequencing.

The study was approved by the ethical committee of the university and written informed consent was obtained from patients or their next of kin to enroll in the study.

Patients were selected from outpatient clinics of the University Hospitals if they were older than 45 years, presenting with gradual onset of cognitive impairment, gait abnormalities, or urinary incontinence, and having magnetic resonance imaging (MRI) brain showing evidence of small vessel disease. There was no upper limit for the patients' age, since cCSVD increases with age.

Patients were excluded if they suffered an acute stroke at any time or if imaging showed more than 50% carotid stenosis. Patients were also excluded if they were diagnosed with any other disorder that may affect white matter or cause the same clinical picture such as demyelinating disease, sarcoidosis, vasculitis, or normal pressure hydrocephalus. Details about family history of similar conditions suggesting CSVD as cognitive decline, gait affection, or urinary incontinence were included in the assessment; however, having a negative family history did not exclude the patients, since we were aiming to assess the genetic load in all CSVD patients.

All patients underwent detailed neurological assessment including past medical history for vascular risk factors and progression of current illness with a focus on family history of similar condition, followed by neurological examination and a battery of tests to assess different aspects of the disease.

These tests included cognitive assessment using the Arabic version of the Montreal Cognitive Assessment Test (MoCA) which has a total score of 30 which signifies normal cognitive functions, while scoring 26 or less implies more cognitive affection [5]. This was followed by gait assessment using a 10-m walk test (10MWT), through self-selected velocity, and fast velocity [6] as well as the Berg balance scale (BBS) which has a score ranging from 0 to 56, calculated out of 14 verbally motoric commands and each is scored from 0 to 4, with 0 indicates least functional ability, while 4 indicates best functional ability with scoring of less than or equal 49 indicates a risk of falls and score beyond 45 indicates more certainty of falls [7].

MRI brain was performed using a 1.5 T MR scanner (Achieva and Ingenia, Philips medical system, Eindhoven, Netherlands) to assess white matter hyperintensity severity using the Fazekas scale [8]. In addition, the SVD score [9] was calculated using 4 MRI markers (white matter hyperintensities, lacunes, cerebral microbleeds, and perivascular spaces; ranging from 0 to 4). The minimum score of 0 denotes the absence of any signs of SVD and the maximum score of 4 implies severe cases with the presence of all four parameters of SVD. Finally, brain atrophy was measured by using the global cortical atrophy scale [10] with a range of 0–39 and a higher grade indicating more atrophy.

For whole exome sequencing: venous blood samples were collected using EDTA tubes and kept at -80 degrees Celsius for later use. Genomic DNA was enzymatically fragmented, and target regions were enriched using DNA capture probes. These regions include approximately 41 Mb of the human coding exome (targeting > 98% of the coding RefSeq from the human genome build GRCh37/hg19), as well as the mitochondrial genome. The generated library was sequenced on an Illumina platform to obtain at least 20 × coverage depth for > 98% of the targeted bases. An in-house bioinformatics pipeline, including read alignment to GRCh37/hg19 genome assembly and revised Cambridge Reference Sequence (rCRS) of the Human Mitochondrial DNA (NC_012920), variant calling, annotation, and comprehensive variant filtering was applied. All variants with minor allele frequency (MAF) of less than 1% in gnomAD database, and disease-causing variants reported in HGMD®, in ClinVar, or in CentoMD® were evaluated.

The investigation for relevant variants was focused on coding exons and flanking ± 10 intronic nucleotides of genes with clear gene–phenotype evidence (based on OMIM® information). All potential patterns for a mode of inheritance were considered. In addition, provided family history and clinical information were used to evaluate identified variants with respect to their pathogenicity and disease causality. Variants were categorized into five classes (pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, and benign) along ACMG guidelines for classification of variants. All relevant variants related to the phenotype of the patient were reported.

Statistical analysis

All data were collected, tabulated in an Excel sheet, and submitted to the Statistical Package for the Social Sciences (SPSS) version 23; data were described as mean and standard deviation (SD) for numerical variables as well as number and percentage for categorical data.

Results

In this cross-sectional study, we investigated patients with insidiously presenting Covert CSVD (cCSVD), as part of a screening project for CSVD genes in Egyptian patients. This disorder seems to be prevalent in our population and was identified as background MRI findings in 63.7% of cases with acute stroke [11].

Thirty patients were recruited presenting with cognitive impairment, gait abnormality, and urinary incontinence, with MRI showing evidence of small vessel disease (SVD). The mean age of the patients was 65.93 years with the mean age of onset at 63.66 years. Male gender represented 63.33% of the studied sample. Cognitive impairment was the main presenting symptom in 18 patients (60% of our cohort). The main risk factors included hypertension found in 63.33% of the cohort, followed by diabetes in 36.66% of the patients. At the same time, family history was positive for similar conditions in only 3 patients as shown in (Table 1).

Whole exome sequencing (WES) was performed for the 30 patients among whom two cases (6.7% of the entire sample) were identified with positive pathogenic variants and their briefing is presented in this article. In contrast, the others were variants of uncertain significance (VUS) (Additional file 1: Table S1).

All patients underwent a battery of tests to assess cognition, gait, and incontinence including the Arabic version of the Montreal Cognitive Assessment (MoCA) [5], and the Berg Balance scale (BBS) [7]. The mean score for MoCA was 17.633 out of 30 and 33.68 out of 56 in BBS, while the mean self-selected speed in the 10-m walk test was 0.4 m/second. The majority of the patients (60%) had a grade of 2 on the Fazekas scale, followed by 33.33% having the highest grade of 3.

Regarding patients with positive pathogenic variants, one patient was a 57-year-old female with uncontrolled hypertension. She has non-consanguineous parents and an older sister with a similar condition who died at the age of 60. She presented insidiously over 5 years with cognitive impairment, gait disturbance, and urinary incontinence, yet no history of strokes or migraine. She scored lower than the mean of the whole sample on motor and cognitive scales (Table 2). Her MRI showed hyperintensities in the temporal poles as well as the external capsule, suggestive of monogenetic SVD (Fig. 1). WES revealed a definite pathogenic variant of typical cysteine changing

Table 1 Characteristics of cCSVD for the whole sample ($n=30$)

Age	Mean ± SD	65.93 ± 8.8	
		n	**Percentage**
Gender	Males	19	63.33%
	Females	11	36.66%
Vascular risk factors	Hypertension	19	63.33%
	Diabetes	11	36.66%
	Dyslipidaemia	10	33.33%
	Current Smoking	7	23.33%
	Ex-smoker	6	20%
	Ischemic heart	5	16.66%
	Family history of similar condition	3	10%
	Sleep apnea	1	3.33%
	Atrial Fibrillation	0	0%
	Previous Stroke	0	0%
First presenting symptom	Cognitive impairment	18	60%
	Gait abnormality	10	33.33%
	Others	2	6.66%
Age of onset	Mean ± SD	63.66 ± 8.8	
Duration of symptoms	Mean ± SD	2.1 ± 1.12	
Fazekas scale	1	2	6.66%
	2	18	60%
	3	10	33.33%
MoCA	Mean ± SD	17.633 ± 4.97	
BBS	Mean ± SD	33.68 ± 12.2	
10m walk SSS	Mean ± SD	0.40 ± 0.11	

SD standard deviation, *MoCA* Montreal Cognitive Assessment, *BBS* Berg Balance Scale, *SSS* self-selected speed

Table 2 Cases with positive pathogenic variants

	Case 1	**Case 2**
Age	57 years	68 years
Gender	Female	Male
Educational level	Secondary grade	Secondary grade
Education years	14	14
Risk factors		
Smoking	No	Yes
Diabetes	No	No
Hypertension	Yes	No
Dyslipidaemia	No	No
ISHD	No	No
Previous CVS	No	No
Family history of similar condition	Yes	No
Age of onset	52	65
Duration of symptoms	5	3
First presenting symptom	**Cognitive impairment**	**Cognitive impairment**
MoCA	11/30	21/30
Berg Balance scale	16/56	32/56
10 MWT (m/sec)	0.25	0.49
ICIQ-SF	18/21	0/21
Fazekas scale	3	2
Small vessel disease scale	3	3
Global cortical atrophy scale	4	22

CVS Cerebrovascular stroke, *MoCA* Montreal Cognitive Assessment, *10MWT* 10-m walk test, *ICIQ-SF* the Arabic International Consultation on Incontinence Questionnaire-Short Form Scale, *SVD* small vessel disease, *ISHD* ischemic heart disease

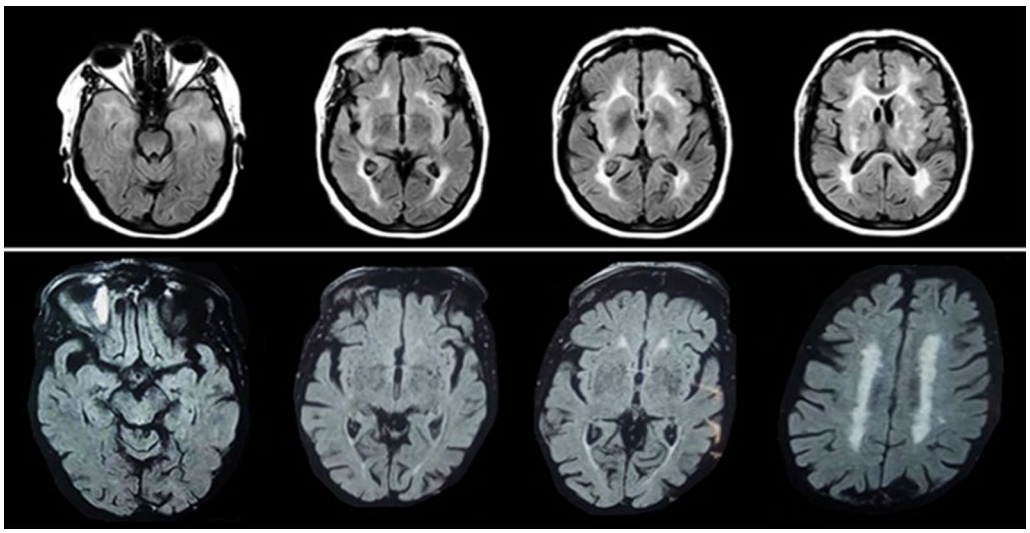

Fig. 1 Upper row showing MRI of case 1 consistent with monogenic SVD. The lower row shows the MRI of case 2 (non-specific findings)

CADASIL mutation in NOTCH3 affecting epidermal growth factor-like repeat 13 (EGFR13).

The second and more interesting patient; was a 68-year-old male. He has no risk factors except for smoking and reports no similar condition in his family.

He presented with a 3-year duration of insidiously progressive cognitive and gait impairment. He scored within the mean values of the whole sample on motor and cognitive scales (Table 2). Although his MRI did not show characteristic features of monogenetic SVD (Fig. 1) yet, WES revealed a definite pathogenic variant COL4A1: p. Pro352Leu.

On the other hand, some patients had mutations or VUS that may need to be further studied for its possible role in the pathogenesis of SVD, as another 60-year-old female patient, known hypertensive and discovered dyslipidemic, with irrelevant family history presenting with a 1-year duration of gait impairment followed by cognitive affection and depressive symptoms. The patient's MRI showed evidence of SVD with Fazekas 3, yet no characteristic pattern. WES showed a mutation in collagen type XXV alpha 1 (COL25A1) which has been previously linked with Alzheimer's disease but never correlated with SVD [12].

One patient had a possibly pathogenic variant in calcium voltage-gated channel subunit alpha 1A (CACNA1A) known to be associated with familial hemiplegic migraine and in close relation to SVD [13], despite the absence of a history of migraines.

Another patient had a mutation in Three Prime Repair Exonuclease 1 (TREX1) and Cathepsin A (CTSA) genes, however, with variants that are most likely benign (Additional file 1: Table S1).

Discussion

It is estimated that cCVSD is responsible for around one-fifth of ischemic strokes as well as a high proportion of hemorrhagic strokes. It is also the main underlying cause found in patients with vascular dementia or vascular cognitive impairment [14].

Many genes have been identified over the years to be responsible for cCSVD such as NOTCH3, HTRA1, COL4A1, COL25A1, TREX1, CTSA, and many others, however, with little data regarding their frequency worldwide. More focus has been on CADASIL patients with NOTCH3 mutations, considered to be the most prevalent monogenic cause of cCSVD and previously estimated to be up to 5 cases per 100,000 individuals [15].

Another large population-based study using the United Kingdom Biobank (UKB) estimated the prevalence of 5 other rare variants (CTSA, TREX1, HTRA1, and COL4A1/2) among 200,000 participants with a phenotype of interest and calculated them to be 0.5% having one or more of these pathogenic variants [16].

To our knowledge, this is the first cCSVD genetic study to be conducted in Egypt and despite the small number of patients, two positive cases were detected representing 6.7% of the studied patients. In comparison with the previously mentioned study, this suggests a huge difference in genetic load between populations. Only one out of three patients with positive family history had a pathogenic variant, while another patient with no family history was also discovered to have a pathogenic variant. This highlights the possibility of monogenic causes in the Egyptian population, even in the absence of a positive family history. Meanwhile, another three cases had VUS that might be linked to cCSVD in future studies.

Despite the absence of therapeutic options for different monogenic cCSVD, the importance of genetic testing relies on diagnosing and categorizing these patients in hopes of developing targeted therapy later on, such as enzyme replacement therapy in patients with Fabry disease [17].

Genetic testing and appropriate counseling can also serve as early biomarkers that can help patients and their families by supplying them with knowledge regarding their illness and arranging for proper lifestyle modification and secondary prevention to prepare for the future [13]. Further studying of these families and similar ones can help in showing the different phenotypes that can be seen with genetic variations, facilitating early suspicion and diagnosis of similar cases.

Conclusion

The current study conducted on a sample of 30 Egyptian patients with cCSVD highlights the underdiagnosis of known monogenetic CSVD in such patients, goes on with the worldwide scientific efforts to link stroke, MRI white matter changes, and SVD to genetic causes, and throws light on possible links of VUS that could be further studied in a larger cohort.

Abbreviations

cCSVD	Covert cerebral small vessel disease
MRI	Magnetic Resonance Imaging
CSVD	Cerebral small vessel disease
CADASIL	Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
NOTCH3	Neurogenic locus homolog protein 3
CARASIL	Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy
HTRA1	High temperature requirement A serine peptidase 1
SVD	Small vessel disease
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
rCRS	Revised Cambridge reference sequence
MAF	Minor allele frequency
HGMD	The Human Gene Mutation Database

OMIM	Online Mendelian Inheritance in Man
ACMG	American College of Medical Genetics
SPSS	Statistical Package for the Social Sciences
WES	Whole exome sequencing
VUS	Variant of uncertain significance
MoCA	Montreal Cognitive Assessment test
BBS	Berg Balance scale
EGFR	Epidermal growth factor-like repeat
COL4A1	Collagen Type IV Alpha 1 Chain
SD	Standard deviation
SSS	Self-selected speed
ISHD	Ischemic heart disease
CVS	Cerebrovascular stroke
10-MWT	10-Meter walk test
BDI	Beck depression inventory
ICIQ-SF	The Arabic International Consultation on Incontinence Questionnaire-Short Form Scale
COL25A1	Collagen type XXV alpha 1
CACNA1A	Calcium voltage-gated channel subunit alpha 1A
TREX1	Three Prime Repair Exonuclease 1
CTSA	Cathepsin A
UKB	United Kingdom Biobank

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41983-024-00810-2.

Additional file 1: Table S1. Seventeen patients with variants of uncertain significance (VUS) representing 56.6% out of the 30 studied cases.

Acknowledgements
This paper is based on work supported by Science. Technology & Innovation Funding Authority (STDF) under grant (38194).

Author contributions
HA: conception of the work, manuscript revision, approval of the version to be published. MM: data collection and research project execution, drafting the manuscript. TR: conception of the work and data collection, manuscript revision, and editing. HS: analysis of data. EH: contribution to the concept and design, drafting the manuscript, analysis, and interpretation of data. BC: interpretation of whole exome sequencing results. HM: interpretation of whole exome sequencing results, drafting the manuscript. MF: contribution to the concept and design, drafting the manuscript. NE: conception and design, revised the manuscript critically for important intellectual content. All authors have agreed to the conditions noted on the Authorship Agreement Form and have read and approved the final version submitted.

Funding
This paper is based on work supported by Science. Technology & Innovation Funding Authority (STDF) under grant (38194).

Availability of data and materials
The corresponding author takes full responsibility for the data, has full access to all of the data; and has the right to publish any and all data separate and apart.

Declarations

Ethics approval and consent to participate
All procedures performed in the study were per the ethical standards of the faculty of medicine, Ain Shams University research and ethical committee, and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. We obtained approval from the Faculty of Medicine, Ain Shams University Research Ethics Committee (FMASU REC) under Federal Wide Association No. FWA 000017585 in 2020. Written informed consent was obtained from participants for participation.

Consent for publication
Not applicable.

Competing interests
None of the authors has any conflict of interest.

Received: 14 November 2023 Accepted: 13 February 2024
Published online: 28 February 2024

References

1. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. Lancet Neurol. 2010;9(7):689–701.
2. Ilinca A, Samuelsson S, Piccinelli P, Soller M, Kristoffersson U, Lindgren AG. A stroke gene panel for whole-exome sequencing. Eur J Hum Genet. 2019;27(2):317–24.
3. Bohlega S, Al Shubili A, Edris A, Alreshaid A, Alkhairallah T, AlSous MW, et al. CADASIL in Arabs: clinical and genetic findings. BMC Med Genet. 2007;9(8):67.
4. Oluwole OJ, Ibrahim H, Garozzo D, Ben Hamouda K, Ismail Mostafa Hassan S, Hegazy AM, et al. Cerebral small vessel disease due to a unique heterozygous *HTRA1* mutation in an African man. Neurol Genet. 2019;6(1): e382.
5. Rahman TT, El Gaafary MM. Montreal Cognitive Assessment Arabic version: reliability and validity prevalence of mild cognitive impairment among elderly attending geriatric clubs in Cairo. Geriatr Gerontol Int. 2009;9(1):54–61.
6. Wolf SL, Catlin PA, Gage K, Gurucharri K, Robertson R, Stephen K. Establishing the reliability and validity of measurements of walking time using the emory functional ambulation profile. Phys Ther. 1999;79(12):1122–33.
7. Berg K. Measuring balance in the elderly: preliminary development of an instrument. Physiother Can. 1989;41(6):304–11.
8. Fazekas F, Kleinert R, Offenbacher H, Schmidt R, Kleinert G, Payer F, et al. Pathologic correlates of incidental MRI white matter signal hyperintensities. Neurology. 1993;43(9):1683–9.
9. Staals J, Makin SD, Doubal FN, Dennis MS, Wardlaw JM. Stroke subtype, vascular risk factors, and total MRI brain small-vessel disease burden. Neurology. 2014;83(14):1228–34.
10. Pasquier F, Leys D, Weerts JG, Mounier-Vehier F, Barkhof F, Scheltens P. Inter- and intraobserver reproducibility of cerebral atrophy assessment on MRI scans with hemispheric infarcts. Eur Neurol. 1996;36(5):268–72.
11. Farag S, Kenawy FF, Shokri HM, Zakaria M, Aref H, Fahmi N, et al. The clinical characteristics of patients with pre-existing leukoaraiosis compared to those without leukoaraiosis in acute ischemic stroke. J Stroke Cerebrovasc Dis. 2021;30(9): 105956.
12. Tong Y, Xu Y, Scearce-Levie K, Ptácek LJ, Fu YH. COL25A1 triggers and promotes Alzheimer's disease-like pathology in vivo. Neurogenetics. 2010;11(1):41–52.
13. Manini A, Pantoni L. Genetic causes of cerebral small vessel diseases. Neurology. 2023;100(16):766–83.
14. Wardlaw JM, Smith C, Dichgans M. Small vessel disease: mechanisms and clinical implications. Lancet Neurol. 2019;18(7):684–96.
15. Haffner C, Malik R, Dichgans M. Genetic factors in cerebral small vessel disease and their impact on stroke and dementia. J Cereb Blood Flow Metab. 2016;36(1):158–71.
16. Ferguson AC, Thrippleton S, Henshall D, et al. Frequency and phenotype associations of rare variants in 5 monogenic cerebral small vessel disease genes in 200,000 UK Biobank participants. Neurol Genet. 2022;8(5): e200015.
17. Wanner C, Germain DP, Hilz MJ, Spada M, Falissard B, Elliott PM. Therapeutic goals in Fabry disease: recommendations of a European expert panel, based on current clinical evidence with enzyme replacement therapy. Mol Genet Metab. 2019;126(3):210–1.

Publisher's Note