Journal of the Neurological Sciences 367 (2016) 239–243

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Phenotypic comparison of individuals with homozygous or heterozygous mutation of *NOTCH3* in a large CADASIL family

Hussam Abou Al-Shaar [a,b], Najeeb Qadi [a,b], Mohamed H. Al-Hamed [c], Brian F. Meyer [c,d], Saeed Bohlega [a,b,*]

[a] Division of Neurology, Department of Neurosciences, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia
[b] College of Medicine, Alfaisal University, Riyadh, Saudi Arabia
[c] Department of Genetics, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia
[d] Saudi Human Genome Program, King Abdulaziz City for Science and Technology, Riyadh, Saudi Arabia

ARTICLE INFO

Article history:
Received 22 September 2015
Received in revised form 10 May 2016
Accepted 31 May 2016
Available online 01 June 2016

Keywords:
CADASIL
NOTCH3
Homozygous
Heterozygous
Mutation
Stroke

ABSTRACT

Background: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a hereditary microangiopathy caused by mutations in *NOTCH3*, very rarely homoallelic.
Objective: To describe the clinical, radiological, and neuropsychological features in an extended CADASIL family including members with either a homozygous or heterozygous *NOTCH3* R1231C mutation.
Methods: The pedigree included 3 generations of a family with 13 affected individuals. The patients were examined clinically and radiologically. Neuropsychological testing was performed on the proband. Sequencing of the entire coding DNA sequence (CDS) and flanking regions of NOTCH3 was undertaken using PCR amplification and direct Sanger sequencing.
Results: Homozygous C3769T mutation, predicting R1231C in exon 22 of *NOTCH3* was found in 7 family members. Six other family members harbored the same in the heterozygous state. Homozygous individuals showed a slightly more severe clinical and radiological phenotype of earlier onset compared to their heterozygous counterparts.
Conclusion: This study reports the largest number of patients with homozygous *NOTCH3* mutation. The phenotype and imaging features of homozygous individuals is within the spectrum of CADASIL, although slightly at the severe end when compared to heterozygotes carrying the same mutation. Both genetic modifiers and environmental factors may play an essential role in modification and alteration of the clinical phenotype and white matter changes among CADASIL patients.

1. Introduction

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an adult onset inherited arteriopathy, characterized by non-hypertensive, non-arteriosclerotic, small arterial granular degeneration. CADASIL manifests as recurrent subcortical ischemic events, progressive or stepwise subcortical dementia, migraine with aura, and mood disorders, with early death [1,2]. Patients usually demonstrate prominent signal abnormalities in subcortical white matter on magnetic resonance imaging (MRI) [3,4]. The mean age of onset of clinical symptoms is the mid-forties; however, MRI abnormalities can be seen a decade before symptom onset [5].

CADASIL symptomatology can be attributed to the systemic vasculopathy encountered in such patients. The smooth muscles in the walls of cerebral arteries are destroyed leading to luminal obliteration and fibrosis, resulting in infarction and stroke [6]. Recurrent infarctions of the white and deep grey matter are the basis of the subsequent dementia and cognitive impairment in these patients.

NOTCH3 is composed of 2321 amino acids. It is a member of a protein family involved in signaling events that control cell fate decisions during development. NOTCH3 is a single-pass transmembrane receptor with a large extracellular domain containing 34 tandem epidermal growth factor-like (EGF-like) repeats [7–9]. NOTCH3 is mainly expressed in smooth muscle cells of small arteries and in pericytes around capillaries [10,11]. There are 33 exons in *NOTCH3* and mutations in this gene are considered to underlie CADASIL. Almost all mutations reported so far are missense mutations that result in a gain or loss of one cysteine residue within an EGF-like repeat domain, with a strong clustering of mutations in exons 4. CADASIL has been reported in various populations around the world [5]. However, only a few cases in the literature reported homozygous mutations of *NOTCH3* [12–16].

* Corresponding author at: Division of Neurology, Department of Neurosciences, King Faisal Specialist Hospital and Research Centre, P.O. Box: 3354, Riyadh 11211, Saudi Arabia.
E-mail addresses: aboualshaar.hussam@gmail.com (H. Abou Al-Shaar), nqadi@kfshrc.edu.sa (N. Qadi), hamed@kfshrc.edu.sa (M.H. Al-Hamed), meyerb@kfshrc.edu.sa (B.F. Meyer), boholega@kfshrc.edu.sa (S. Bohlega).

http://dx.doi.org/10.1016/j.jns.2016.05.061
0022-510X/

Herein, we report the largest number of homoallelic cases of CADASIL. We describe and compare the phenotypes of homozygous and heterozygous members within this family.

2. Methods

2.1. Patients

Thirteen affected individuals from a 3 generation family were enrolled for this study. All were examined in the Department of Neurosciences, King Faisal Specialist Hospital and Research Center (KFSH&RC). The protocols for this study were approved by the institutional review board and informed consent was obtained from all participants. All subjects were enrolled under an IRB-approved protocol (RAC# 2020023) with full informed consent.

History was taken and clinical examination performed on the index case and his family members. MRI was obtained for all patients. Neuropsychological analysis was performed for the index case alone.

2.2. NOTCH3 sanger sequencing

DNA was isolated from whole blood using a standard salt precipitation method using a Gentra Puregene blood kit (Qiagen). Sequencing of the entire coding and flanking regions of *NOTCH3* was undertaken using PCR amplification and direct Sanger sequencing using a BigDye terminator kit (Thermo Fisher, Foster City, CA). SeqScape v.2.6 software (Thermo Fisher, Foster City, CA) was used to align sequence data with *NOTCH3* (ENSG00000074181).

3. Results

3.1. Clinical history

The family reported in this paper originated from Kashmir in the North Eastern part of the Indian sub-continent and included 13 affected individuals spanning 3 generations as described below (Table 1). Of note, the last generation is a product of triple loop consanguinity as seen in Fig. 1.

3.1.1. IV-53 homozygous patient (as indicated in Fig. 1)

The index case is a male practicing physician. He was well until the age of 53 when he had an acute onset right-sided hemiparesis, which left him with some clumsiness. Six months later, he suffered from numbness affecting the left side. Afterwards, he had problems with his vision, which he described as nocturnal blindness worse in the left eye. Ophthalmological examination showed arteriovenous nicking with moderate compression of the retinal veins as well as retinal microinfarction and chorioretinal scaring temporal to the left macula. There were tortuous narrowed retinal arterioles with mild optic disc cupping. Subsequently, multiple strokes occurred over nine years of follow up, which left him with progressive dysarthria and a spastic gait. He became easily fatigued and markedly depressed. He was mentally slow but was able to continue his work as a Pediatrician in the Emergency Room. His MRI showed diffuse bilateral lacunar infarcts affecting basal ganglia, centrum semiovale, and periventricular white matter (Fig. 2A). Carotid angiography at the age of 55 was normal. The cognitive performance at the age of 59 (six years after the first stroke) showed a Mini-Mental Status Examination score of 24. Neuropsychology testing revealed mild problems with executive function, verbal abstraction, Wechsler Adult Intelligence Scale with visuospatial ability measured by Rey-Osterrieth Complex Figure copying and immediate reproduction and 12-memory test with free recall (12 word Rec) and recognition. Slight bipolar mood disorder was also moderately observed. The disease progressed relentlessly in a stepwise and accelerated pattern. The patient experienced difficulty swallowing associated with a pseudo bulbar affect, progressive dementia, and severe depression. He was admitted several times during this period with repeated chest infection until he became severely disabled for more than a year prior to his death.

His father (III-78) died at the age of 78 after 9 years of repeated strokes, dementia, impaired vision, and moderate hearing loss. His symptoms started at the age of 62, however, he was not properly investigated. The index case's mother (III-84) died at the age of 84. She had asymmetrical coarse tremors and bradykinesia. She was diagnosed with Parkinson's disease during her last few years and she responded well to Levodopa therapy.

3.1.2. III-66 heterozygous patient

The paternal uncle (Father-in-Law) of the index case was initially seen at the age of 73 with history of 3 repeated strokes, which started at the age of 66. His brain computed tomography (CT) scan was done at the age of 69 and repeated three years later during which he was diagnosed with Binswanger's disease with progression of the Leukoaraiosis. Brain MRI showed diffuse white matter hyperintense ischemic changes. He was moderately demented with hyper-reflexia and pseudo-bulbar affect.

3.1.3. III-76 heterozygous patient

Paternal Aunt (Mother-in-Law) was asymptomatic at the age of 76 but brain MRI showed a few ischemic lesions in the basal ganglia and deep white matter (Fig. 2C).

3.1.4. IV-36 homozygous patient

Sister of the index case started to experience recurrent episodes of migraine without aura at the age of 36. Repeated episodes of asymmetrical weakness and numbness started at the age of 42 indicating vascular insult (stroke). She also suffered from progressive visual difficulty with ocular transient ischemic attacks with retinal microinfarcts and hemorrhages. At age of 52, she started experiencing difficulty walking followed by dementia, night blindness, and seizure disorder. She

Table 1
Clinical, radiological, and exon 22 C3769T (R1231C) mutation status of the patients in relation to the index case.

No.	Age of onset (years)	Sex	Relation to index case (Fig. 1)	Clinical picture	MRI	Genotype C>T
III-76	76	F	Paternal Aunt (Mother-in-Law)	Asymptomatic	Abnormal	c/t
III-66	66	M	Paternal Uncle (Father-in-Law)	Stroke and dementia	Abnormal	c/t
IV-35	35	M	Cousin	Migraine with aura	Abnormal	t/t
IV-50	50	M	Cousin	Asymptomatic	Not done	c/t
IV-38	38	F	Wife	Migraine with aura	Abnormal	t/t
IV-53	53	M	Index case	Stroke	Abnormal	t/t
IV-36	36	F	Sister	Migraine without aura, stroke, dementia, seizure, and night blindness	Abnormal	t/t
V-7	7	F	Daughter of the second wife	Asymptomatic	Not done	c/t
V-11	11	M	Son of the second wife	Asymptomatic	Not done	c/t
V-13	13	F	Daughter of the second wife	Asymptomatic	Not done	c/t
V-22	22	M	Son	Asymptomatic	Abnormal	t/t
V-30	30	M	Son	Asymptomatic	Abnormal	t/t
V-31	31	F	Daughter	Asymptomatic	Abnormal	t/t