Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy in Arabs

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ABSTRACT

الأهداف: تحري الطفرات الجينية الموجودة في جين Notch 3 في مرض الكداسيل CADASIL عند العرب. مرض كداسيل CADASIL هو اعتلال الشرايين السائد يصاحبة جلطات دماغية مع اعتلال بيضاء الدماغ، وهو مرض وراثي، دماغي، وعائي موصوف باضرية إقفارية تحت قشرة المخ، وغالبا ما يبدأ ظهور أعراض هذا المرض في العقد الثالث أو الرابع من العمر.

الطريقة: تم إجراء فحص عصبي شامل مع فك الشفرة الوراثية لجين Notch 3، في مستشفى الملك فيصل التخصصي ومركز الأبحاث – الرياض – المملكة العربية السعودية، خلال عام 2007م، لعائلتين إحداهن من المملكة العربية السعودية، والأخرى من السودان مصابه بهذا المرض.

النتائج: كان لدى الحالات المصابة ضربة أولية بالغة، مع خرف وعائي، وأعراض سلوكية ونفسية عجلت بالوفاة. الفحص بالرنين المغناطيسي أظهر إصابات بالمادة البيضاء عند المصابين وغير المصابين. فحص التسلسل للقواعد النيتروجينية في جين Notch 3 لم يظهر أي طفرات جينية في هذا الجين.

خاتمة: تعتبر هذه الدراسة أول وصف لمرض كداسيل CADASIL عند العرب بدون وجود طفرة جينية في جين Notch 3.

Objectives: To investigate the Notch 3 mutation spectrum in Arab patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), which is an inherited cerebrovascular disease characterized by recurrent subcortical ischemic stroke starting in the third or fourth decade.

Methods: Complete neurological evaluation and sequencing of the Notch 3 gene were carried out at King Faisal Specialist Hospital & Research Centre in 2007 on 2 families from Riyadh, Kingdom of Saudi Arabia and Sudan affected by CADASIL.

Results: The index cases had adult onset stroke, vascular dementia, behavioral and psychiatric symptoms and accelerated deaths. In both families, abnormal magnetic resonance imaging findings were detected in symptomatic and asymptomatic individuals. All Notch 3 exons were screened for mutations in both families and no known or novel mutation could be found; although, in one family the brain biopsy showed the typical granular osmiophilic material deposition and the vascular smooth muscle cells.

Conclusion: This is the first 2 cases of CADASIL in Arabs, which occur without an obvious Notch 3 mutation.

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Cerebral autosomal dominant arteriopathy with subcortical infarct and leukoencephalopathy (CADASIL) is an adult onset inherited arterial disease of the brain, with non-hypertensive, non-arteriosclerotic, small arterial granular degeneration; characterized by recurrent subcortical ischemic events, progressive or stepwise subcortical dementia, migraine with aura, and mood disorders, with early death.[1,2] All symptomatic individuals had prominent signal abnormalities in subcortical white matter on magnetic resonance imaging (MRI).[3] The mean age of onset of clinical symptoms is the mid-40s,[4] however, MRI abnormalities can be seen a decade before symptoms appear.[5] Pathological features are systemic vasculopathy, predominantly involving the smooth muscle cells of the small cerebral anterials.[5,6] Electromicroscopy study may demonstrate deposits of granular osmiophilic material (GOM) which is pathognomic of the disease. The Notch

3 genes encode Notch 3 protein of 2321 amino acids and mutations in this gene have been identified as the genetic cause of CADASIL. The Notch 3 is a member family which is involved in signaling events that could control cell fate decisions during development. It is a single-pass transmembrane receptor with a large extracellular domain containing 34 tandem epidermal growth factor-like (EGF-like) repeats.[7] There are 33 exons in the Notch 3 gene and mutations reported thus far results in a gain or loss of one cysteine residue within an EGF-like repeat domain and with a strong clustering of mutations in exons 3 and 4.[8] Cerebral autosomal dominant arteriopathy with subcortical infarct and leukoencephalopathy have been reported in various populations round the world [9-13] and recently we reported CADASIL occurrence in 3 Arab families with mutations in the Notch 3 gene.[14] In this study, we are presenting 2 additional Arab families with similar clinical picture to our previously reported cases, but with no obvious mutation in the Notch 3 gene.

Methods. Complete neurological evaluation and sequencing of the Notch 3 gene were carried out at King Faisal Specialist Hospital & Research Centre in 2007 on 2 families from Riyadh, Kingdom of Saudi Arabia and the Sudan affected by CADASIL.

Family enrollment. Requests to identify cases and participants in this study were carried out through mailing a brief inclusion criteria form for the disease to all members of the Pan Arab Union of Neurological Sciences (PAUNS). Families were included when an index case had both a history of transient ischemic attacks (TIA) or subcortical stroke of unidentified etiology, positive family history of stroke with early death or dementia compatible with autosomal dominant traits, and a cranial MRI scan showing diffuse or focal microangiopathic white matter abnormalities. Two families with pure Arabic ethnic background fulfilled the inclusion criteria. Family A was from Saudi Arabia, whereas family B was from the Sudan.

Clinical assessment. All subjects, their siblings and other available family members (**Figure 1**) have had detailed clinical evaluations and assessment of risk factors. Previous and current imaging results were collected. In one family (Family B) MRI brain scans were performed in all sib ships of the index case. The proband in Family B was diagnosed as possible CNS angiitis, so brain, and meningeal biopsy were performed and showed small vessels angiopathy with multiple osmiophilic, granular electron-dense material without atherosclerosis or amyloid deposition (**Figure 3**).

Sample collection and DNA extractions. Five milliliters of peripheral blood were collected in EDTA tubes from all participating individuals after obtaining their consent. Deoxyribonucleic acid was extracted from whole blood samples of all CADASIL patients and their family members using the PURGENE DNA isolation kit from Gentra Systems (Minneapolis, USA).

Mutation analysis of the Notch 3 gene. DNA from patients and their families were amplified using primers designed to amplify the 33 exons, including the intron-exon boundaries, of the Notch 3 gene. The same amplification primers were also used for sequencing. Sequencing was carried out using the Dynamic Terminator Reagent Sequencing kit [Amersham Pharamcia, US 81090]. The samples were then run on the DNA analyzer [MegaBACE 1000 Capillary system; Molecular Dynamics, Amersham Pharmacia Biotech]. Data from the analyzer were analyzed using the Chromas Pro version 1.34 (Technelysium, Pty, Ltd, Australia). We also investigated the frequency of each detected mutation in our normal controls Primers, and PCR conditions used for screening the Notch 3 gene were described previously.[8]

Results. ***Family A.*** The proband had repeated strokes around the age of 32, leading to severe disability and subcortical dementia at age 45, screening for diabetes, hypertension, and vasculitis were negative. The father died at age 62 with multiple strokes (**Figure 1a**). One sibling had behavioral problems at age 32. Magnetic resonance imaging brain showed white matter disease in 2 asymptomatic siblings at the age of 30 and 38 (**Figure 2a-2d**). Direct sequencing of the 33 exons of the Notch 3 gene did not show any previously reported or novel mutation(s).

Family B. The index case in this Sudanese family was seen at age 49, with a 2 years history of repeated episodes of right leg numbness, weakness, and dysarthria. His father died at the age of 66 with strokes and dementia. Two out of 6 siblings had multiple strokes in their early fifties (**Figure 1b**). There was no history of diabetes, smoking, or hypertension. Diagnosis of possible CNS vasculitis was entertained, and patients went for brain and meningeal biopsy. Parynchymal vessels biopsied from the brain showed granular material positive to Periodic Acid Schiff and characteristic GOM was seen on electron microscopy (**Figure 3**). Magnetic resonance imaging for all 4 patients showed multiple deep white matter lesions. (**Figure 2e & 2f**) Direct sequencing of the 33 exons of the NOTCH3 gene did not show any previously reported or novel mutation(s).

Discussion. The inheritance pattern in these 2 families is clearly autosomal dominant, and the patients exhibited a variety of characteristics that are typical of CADASIL, such as: migraine, recurrent strokes, vascular dementia, depression, and behavioral problems. All of the

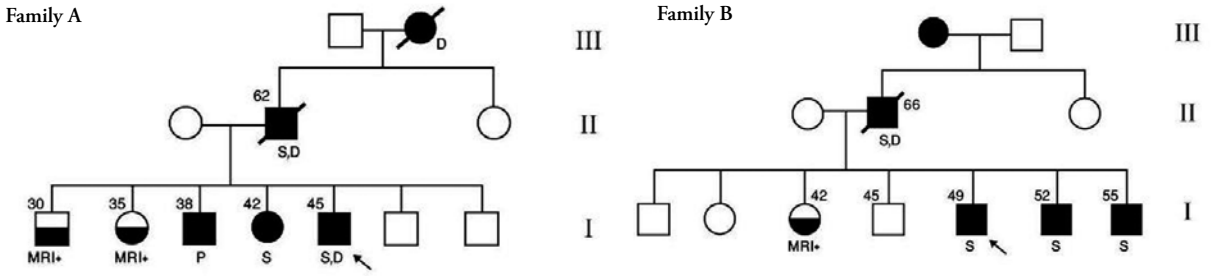

Figure 1 - Pedigree of the 2 Arabs studied kindreds. S: stroke, D: dementia, M: migraine, P: psychiatric manifestations. Number(s) at the top indicates age at death or at time of examination. **a**) Diagonal fill indicates asymptomatic positive for mutations, **b**) horizontally fill indicates asymptomatic individual with abnormal brain MRI.

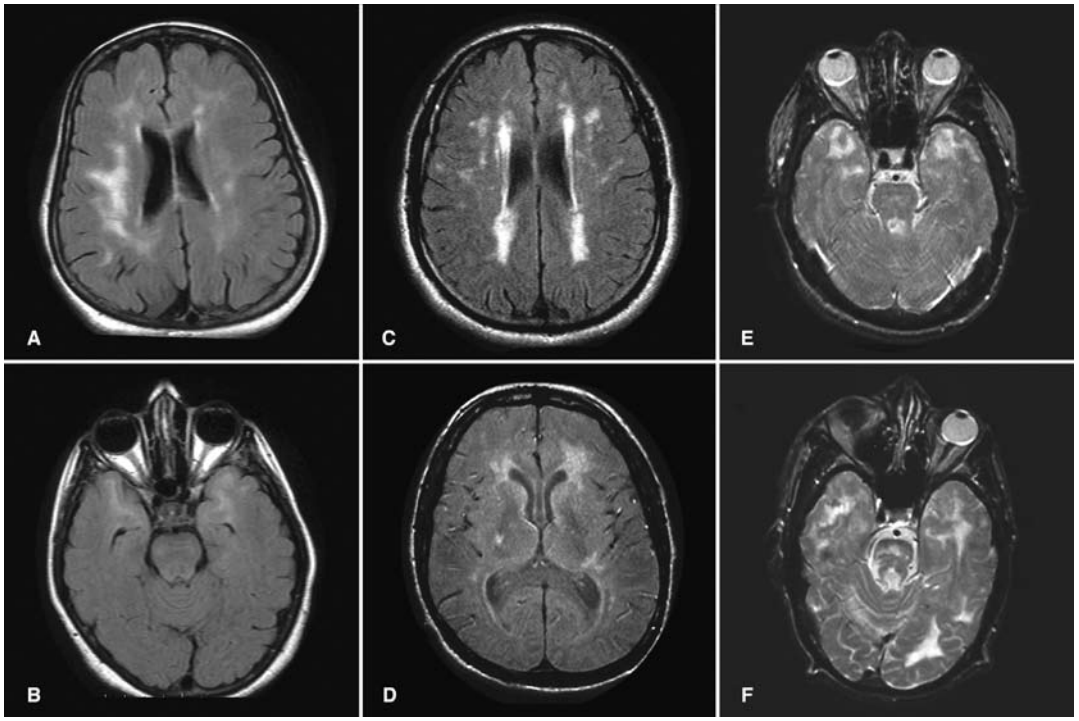

Figure 2 - Magnetic resonance imaging showing the **A-D**) Axial and T2 weighted **E & F**) MRI from patients showing bilateral periventricular diffuse white matter ischemic lesion and multiple lacunar lesions in thalamus, pons, and basal ganglia. The exams in A and B are from asymptomatic patients with depression and behavioral problems; E and F are from index patient and his father (Family B). Note temporal lobe lesions even in asymptomatic patients (B).

affected members in the several generations died age 45-55 years as a result of recurrent strokes. All symptomatic individuals also had prominent signal abnormalities in subcortical white matter on MRI affecting temporal lobes and external capsule (**Figure 2**). Abnormalities in brain MRI were observed in asymptomatic individuals indicating subclinical strokes.[5] In addition, presenting symptoms noted at different ages and the members from the same family may have different clinical presentation (**Figure 1**).[6] It has been suggested that CADASIL is commonly misdiagnosed and proportionally under diagnosed because of its pleiotropic symptoms such as CNS vasculitis, infection or immune mediated vascular disorders because it can be present under various guises.[2,6] The failure to detect CADASIL as a common cause of vascular dementia or stroke worldwide may reflect geographical variations and pathogenetic influences rather than simple under-recognition.[15,16] The phenotype, MRI, and pathological features are difficult to overlook as in the case in our 2 families. Diagnosis of CADASIL can be approached pathologically; vascular

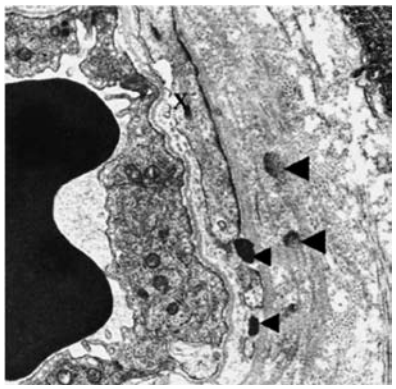

Figure 2 - Electron microscopy picture from the meningeal arteriole. There is abundant granular osmiophilic material arrowhead on the surface of the vascular smooth muscle cell or between the separated basal laminae (indicated by "X").

GOM can be seen on electron microscopy of the skin, muscles, and peripheral nerves.[5] Although skin biopsy is practical, patients can have false negative results.[6,7] Recently, an immunostaining technique was developed that uses murine monoclonal antibodies 1E4 raised against EGF-like repeats. Confirmatory studies show that the skin biopsy immunostaining technique has a high sensitivity (96%) and specificity (100%) for diagnosis of CADASIL.[8,17] Our mutation-negative family (Family B) had the characteristic vascular deposits, GOM seen in electron microscopy and clear positive family history.[17] All members studied exhibited typical clinical and MRI findings so we considered it unlikely that these patients' conditions had been misdiagnosed.

Genetic studies for CADASIL are associated with a noticeable proportion of false-negative results, despite performing direct sequencing of exon 2 through 24 of the Notch 3 gene.[5,15] Recently false-negative results were seen in 5 families (4%) of biopsy-proven CADASIL.[15] All CADASIL related mutations reported thus far occur in exons that encode one of the 34 EGF repeat domains. To date, approximately 150 different mutations in the Notch 3 gene have been reported in CADASIL patients. According to the Human Gene Mutation database (HGMD), Notch 3 mutations associated with CADASIL are as follows: 144 missense/nonsense point mutations, 2 splicing mutations, 5 small deletions, and one gross deletion.[16] In these 2 Arab families, no Notch 3 mutations could be detected after sequencing the full gene. This is not unusual as Joutel and others could not detect any mutation in 5 patients with typical clinical picture of CADASIL.[4] It is possible that these 2 families may carry a point mutation within the non-coding region of the gene or gross rearrangements of Notch 3, such as deletions, which are unlikely to be detected by PCR-based screening used here. It is also conceivable that polymorphisms or mutations in other genes known to regulate expression, trafficking, and activity of the Notch receptor genes may contribute to the development of CADASIL in the absence of Notch 3 gene mutations.[18] The possibility that our families and other families reported in the literature[19,20] may represent a novel disorder differently from Notch 3 causing CADASIL has been entertained previously[19,20] and further genetic clarification is required.

In conclusion, these 2 families add insight into the difficulties of diagnosing CADASIL on a clinical basis. Mutation analysis and pathological findings prone to be a valuable tool for diagnosing this disorder, both in confirming the diagnosis and broadening of our knowledge of the clinical variations and phenotypic spectrum of this disease as well as confirming or disproving its genetic homogenicity and hopefully produce further research into pathophysiology of CADASIL syndrome.

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