Abstract  Here we describe clinical, neuropsychological and neuroradiological findings in 6 subjects belonging to two unrelated Italian cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) kindreds from the same geographic area who shared a common Arg1006Cys mutation. Subjects from Family A were virtually asymptomatic, and yet showed MRI pathological findings and a cluster of sub-clinical neuropsychological defects mainly centred on the visuospatial domain; patients from Family B had presented several clinically relevant episodes and showed a general cognitive impairment compatible with the clinical picture of vascular dementia. The present clinical observations are consistent with the hypothesis of a geographical clustering for CADASIL, and highlight that sub-clinical cognitive impairment may help to identify this syndrome in families presenting with only migraine.

Key words  CADASIL • Cognitive impairment • Genetics • Neuropsychology • Vascular dementia

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a genetic disorder characterised by systemic vascular abnormalities, diffuse white matter lesions and small subcortical infarcts on neuroimaging [1]. Its clinical manifestation results from variable combination of recurrent cerebral ischaemic episodes (TIA or stroke), cognitive deficits, migraine with aura and psychiatric symptoms [2]. The pathological hallmark of CADASIL is a granular osmiophilic material (GOM) within the basal membranes of smooth muscle cells of cerebral [3] and extracerebral arterioles [4]. The Notch3 gene, which includes 33 exons encoding a protein of 2321 amino acids, has been identified as the defective gene in CADASIL [5].

CADASIL was initially thought to be a rare disorder, but increasing numbers of families have been reported world-wide. The diagnosis of CADASIL is often difficult because clinical and neuroimaging features lack specificity; therefore, diagnosis rests on genetic confirmation. However, the Notch3 gene has a large number of exons and CADASIL has a broad mutation spectrum [6]; therefore, planning of genetic screening would greatly benefit from information about geographic distribution of mutations [7]. Here we describe two unrelated Italian kindreds from the same geographic area who shared a common Arg1006Cys mutation.

Materials and methods

Subjects

Two patients belonging to two families from a small village (Appignano) in Central Italy came to our observation (Division of Neurology, Mazzoni Hospital, Ascoli Piceno) because of persistent migraine (Family A, member III:9) and progressive cognitive and motor impairment (Family B, member III:1). The two families were not related; their pedigrees are shown in Figure 1.
On the basis of clinical and neuroradiological findings we suspected CADASIL and decided to evaluate other individuals from both kindreds. Twelve subjects, 8 from family A (7 males, 1 female, age range 53–67) and 4 from family B (3 males, 1 female, age range 45–73), gave their informed consent to participate in the study, approved by the Mazzoni Hospital ethics committee. Clinical information about dead subjects was collected from relatives.

Molecular analysis

Genomic DNA was extracted from peripheral blood leukocytes of all 12 subjects with a standard salting out procedure. Exons 3, 4, 11, 19 and 8, which could account for up to 90% of CADASIL cases [8], were analysed by automated direct nucleotide sequencing using an ALF express automated DNA sequencer (Amersham Biosciences). Oligonucleotides for polymerase chain amplification (PCR) were designed following the Notch3 genomic clone accessible in the NCBI database (http://www.ncbi.nih.gov; accession number AF058881) and are available on request; for each PCR reaction, one oligonucleotide was biotinylated; the template DNA strand was immobilised by streptavidin and sequenced by internal Cy5-fluorescently labelled oligonucleotides using the Autoload Solid Phase Sequencing kit (Amersham Biosciences).

Neuropsychological assessment

The neuropsychological evaluation was performed by means of tests already used in previous studies in CADASIL [8], plus other additional tests. Tests were chosen to assess general cognitive performance, selective attention and information processing, abstract thinking, verbal and spatial short-term memory, verbal learning, verbal comprehension, and constructional, limb and oral praxis. All tests were given in their Italian versions that allow discrimination between normal and pathological performances on the basis of age- and education-adjusted scores.

Results

Six out of 12 subjects, 3 belonging to Family A (III:3, III:6 and III:9) and 3 (II:1, II:4 and III:1) to Family B, harboured a heterozygous Cgc to Tgc transition at codon 1006 in the Notch3 exon 19, as already reported in CADASIL patients [7, 9]. The mutation substituted an arginine with a cysteine (Arg1006Cys) in the 26th epidermal growth factor (EGF)-like domain of the Notch3 extracellular domain.

All subjects had a variable degree of diffuse leukoencephalopathy and small subcortical infarcts on neuroradiological investigation, with ventricular enlargement in symptomatic patients from Family B. Neuropsychological tests (Table 1) revealed several pathological scores in all of them. Here we provide brief sketches of the clinical history of the six patients.

Family A

Family members (FM) III:3 and III:6
At age 65 and 66 these subjects did not show pyramidal or extrapyramidal signs or symptoms. They had no history of depression, psychiatric disturbances or migraine; in the