A Novel NOTCH3 Gene Mutation in a Polish CADASIL Family

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Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) is a genetically determined disease of the cerebral vessels, characterized by recurrent ischemic strokes, dementia, and degeneration of the cerebral white matter. The condition is caused by a mutation in the NOTCH3 gene, whose product plays a great role in the development and physiology of the cardiovascular system. Magnetic resonance imaging reveals multiple hyperintensive lesions of the white matter in the T2-weighted images also in asymptomatic carriers of CADASIL and can be detected even 10-15 years prior to clinical signs. Diagnosis is confirmed by genetic testing. We present 2 patients (mother and daughter) carrying the same mutation p.Cys212Gly in 1 allele of the NOTCH-3 gene, which has not yet been recorded in the Human Gene Mutation Database for that gene and therefore described as a new one. The clinical manifestation of the disease differs between patients −the 63-year-old mother has been suffering from severe migraine headaches since her early youth and the first vascular event occurred when she was about 50 years old, she is now presenting with impaired cognitive functions, left facial palsy, bilateral pyramidal syndrome more prominent on the left side, and four-wheel support assisted walking. The neurological deficits that her 42-year-old daughter is afflicted with are discreet. Observation to date indicates a definitely less severe clinical course of the disease. This indicates that members of the same family carrying the same mutation may produce different clinical course of the disease.

Key Words: CADASIL—NOTCH3—novel mutation

Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) is a genetically conditioned disease of the cerebral vessels characterized by recurrent ischemic strokes, dementia, and degeneration of the cerebral white matter.[1,2]

We present 2 patients (mother and daughter) carrying the same mutation p.Cys212Gly in 1 allele of the NOTCH-3 gene, which has not yet been recorded in the Human Gene Mutation Database (HGMD) for that gene and therefore described as a new one.

Case Report

A 63-year-old female with a history of migraine headaches dating back to her childhood was later diagnosed with arterial hypertension, dyslipidemia, and hypothyroidism resulting from previously performed thyroidectomy procedure. At the age of 50 she suffered the first epileptic seizure. She was admitted to the neurological department, where she was diagnosed with epilepsy. Five years later, at 50, she was admitted to our department with progressive bilateral hemiparesis. A magnetic

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resonance imaging (MRI) revealed the presence of vascular foci in the periventricular area and the corpus callosum as well as cortical atrophy most prominent in the temporal lobes. She was diagnosed with small vessel disease probably caused by arterial hypertension. A year later, she was again admitted to our department with aggravated left-side hemiparesis. The presence of vascular encephalopathy and symptomatic epilepsy indicated that the woman could be afflicted with MELAS syndrome, which, however, was not confirmed by further investigations. Her subsequent hospital admission was 7 years later and was due to a protracted epileptic clouded state following generalized tonic clonic seizure. On admission the patient was in logical verbal contact with stable cardiovascular and respiratory status. She was diagnosed with impaired cognitive functions, left facial palsy bilateral pyramidal syndrome, more prominent on the left side, and four-wheel support assisted walking. Computed tomography examination performed on admission showed numerous lacunar strokes located in deep structures of the cerebrum, predominantly in the grey matter of the basal ganglia, both parts of the thalamus and the right portion of the pons. Diffused extensive hypodense areas mainly of periventricular location, generalized cerebral cortical atrophy particularly deep in the temporal lobes were also found. The diagnostics was extended by a contrast enhanced MRI (17. 06. 2016) which confirmed the presence of numerous lacunar foci and advanced degeneration of the periventricular white matter (Fig 1).

A video-EEG study showed slow waves from the frontotemporal area with lateralization of the left-hemispheric dominance and tendency to generalization.

A carotid and vertebral Doppler ultrasonography demonstrated a normal spectrum of blood flow. Neuropsychological assessment revealed cognitive impairment (23 points on the MMSE scale, having regard to age, education, a reduced auditory, and visual immediate memory span, impaired working memory and lower verbal fluency). Taking into consideration the clinical data, the patient was suspected to suffer from CADASIL. A genetic test showed a mutation of p.Cys212Gly in 1 allele of the *NOTCH-3* gene. Due to the positive result of the test there was no need for a skin-muscle biopsy.

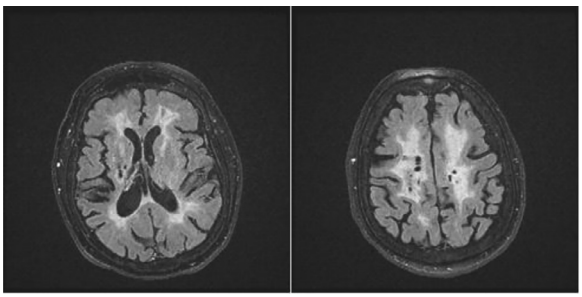

Figure 1. *The patient's T2-weighted FLAIR brain MRI images - numerous lacunar foci and advanced degeneration of the periventricular white matter.*

The woman's 3 daughters (aged 28, 37, and 42) were tested for relevant genetic abnormalities. The 42-year-old one was found to have an identical mutation of p. Cys212Gly in 1 allele of the *NOTCH-3* gene. No mutations were detected in the other daughters.

At the time of the examination (March 2017) the daughter affected with the mutation did not show any clinical manifestations. Three years earlier she experienced a transient episode of speech disturbance and a sensation of numbness in the right limbs, which occurred during a puerperal period (2 months after the delivery). No neurological diagnostic tests were carried out then. Having obtained positive results of the genetic tests confirming that the woman carried a mutation in the *NOTCH-3* gene, we referred her for an MRI, which revealed numerous vascular foci in the white matter of both hemispheres, which partially fused in the region of both posterior horns of lateral ventricles and an isolated lacunar focus in the left external capsule (Fig 2).

Four months later (02.10.2017) the daughter suffered a transient episode of cerebral ischemia which caused double vision while looking sideways, particularly to the right. She also presented with a discrete right-sided facial palsy, bilateral hyperreflexia more prominent on the left side with no pathological signs manifested.

A follow-up MRI scan did not reveal any new ischemic foci (image comparable to the one obtained at a previous examination in March). The complaints she reported on admission resolved completely on her second hospital day. It was concluded that the woman suffered a CADASIL-induced transient episode of cerebral ischemia. She was prescribed acetylsalicylic acid and pentoxifylline derivatives as secondary prevention for cerebral vascular accidents.

Discussion

Our CADASIL patients carry mutation p.Cys212Gly in 1 allele of the *NOTCH-3* gene, which has not yet been recorded in the HGMD for that gene and therefore described as a new one. However, the database does contain a record of 3 other mutations caused by amino acid substitution of cysteine from position 212 of the protein

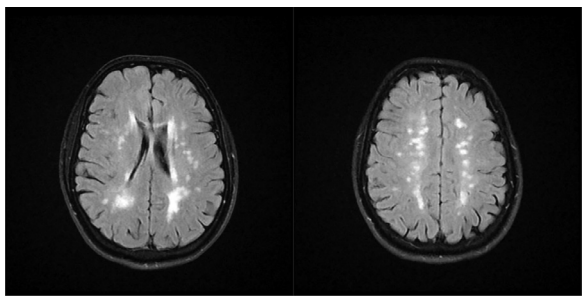

Figure 2. *The daughter's T2-weighted FLAIR brain MRI images showing numerous vascular foci in the white matter of both hemispheres fusing partially near both posterior horns of lateral ventricles and a single lacunar focus in the left external capsule.*

coded for by the *NOTCH-3* gene by amino acids other than glycine. The coding section (exons 4 and 5) of the *NOTCH-3* gene together with the sequences surrounding the exons were studied using a DNA sequencing method.

The mother has been suffering from severe migraine headaches since her early youth and the first vascular event occurred when she was about 50 years old. Now (Oct. 2017) the woman needs the four-wheel support to walk.

The neurological deficits her 42-year-old daughter is afflicted with are discreet despite the pathological change in the brain revealed through the MRI examination. Observation to date indicates a definitely less severe clinical course of the disease. Documented studies of families affected with CADASIL also demonstrate different courses of the disease in subjects carrying the same mutation, including monozygotic twins, which implies the role of environmental factors.[3,4]

A multi-centre retrospective study on Chinese population provided evidence that CADASIL is a syndrome of genotypic and phenotypic heterogeneity. Severity of clinical manifestations and their onset was closely correlated with a type of the mutation as well as its location in particular exons. In 35% of the subjects mutations p.Arg607Cys or p.Arg544Cys occurred in exon 11. Carriers of these mutations were more susceptible to cognitive disorders, significantly lower incidence of migraine with aura and an older age of the onset.[5] Similarly, members of the same family may produce different clinical course of the disease.[6,7]

The phenotypic image of the neurological disorders present in the family we have described does not differ significantly from other descriptions. The daughter burdened with mutation has not yet presented symptoms corresponding in severity with the changes revealed through the MRI or the extent of her mother's deficits. It should be noted though that her mother's onset age was about 50 years and the daughter is just 42.

The most frequently described missense mutation results in addition or deletion of cysteine residue predominantly in exon 4.

Research is being done on potential causal treatment options such as the application of low-molecular-weight thioflavin T derivatives, DAPH (4,5-dianilinophtalimide) i SA (staurosporine aglycone), reducing the number of the NOTCH 3 protein deposits. A prospect of a genetic therapy consisting in the correction of a mutation using oligonucleotides for placing an additional molecule of cysteine into a mutant protein molecule is also being investigated.[8,9,10]

Despite the lack of "specific" treatment, an early diagnosis of CADASIL, even in asymptomatic carriers, is beneficial as it provides an opportunity for lifestyle change and elimination of a number of cardiovascular risk factors like smoking tobacco, hypertension, or dyslipidemia.[9,11]

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