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Disease progression and high field MRI in CADASIL

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Chapter 6

Homozygosity for a NOTCH3 mutation in a 65 year old CADASIL patient with mild symptoms: A family report

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ABSTRACT

Background

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is caused by mutations in the NOTCH3 gene and is clinically characterized by recurrent stroke and cognitive decline. One patient has been described with a homozygous NOTCH3 mutation to date. This patient was at the severe end of the clinical spectrum of CADASIL. The question remains whether or not a homozygous mutation is associated with a more severe clinical phenotype in CADASIL.

Methods

We studied a Dutch patient with a homozygous NOTCH3 mutation (Arg578Cys), using standardized neuropsychological and MRI examinations, and compared her clinical phenotype to a brother with a heterozygous mutation and a sister without a mutation. Homozygosity of the NOTCH3 mutation was confirmed with MLPA.

Results

The homozygous patient had her first stroke at age 64 and had no cognitive dysfunction. Her MRI scan revealed white matter hyperintensities, lacunar infarcts and microbleeds consistent with CADASIL at this age. MR Spectroscopy revealed a lactate peak. Her heterozygous brother had his first stroke at age 67 and had no cognitive dysfunction. His MRI examination showed white matter hyperintensities and no lacunar infarcts or microbleeds. Diffusion Tensor Imaging measurements showed similar values of Apparent Diffusion Coefficient and Fractional Anisotropy in both patients.

Conclusion

The clinical phenotype of both our homozygous patient and her heterozygous brother is at the better end of the spectrum of CADASIL. This provides evidence that heterozygous and homozygous CADASIL patients are clinically indistinguishable.

INTRODUCTION

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a hereditary microangiopathy caused by mutations in the *NOTCH3* gene.(1) The microvascular changes are systemic, but the cerebral vasculature carries the brunt of the pathology.(2) Clinical hallmarks are recurrent stroke, cognitive decline, migraine with aura (in up to 40% of patients), and mood disorders (up to 30%).(3) MRI invariably reveals characteristic white matter hyperintensities (WMHs) with or without lacunar infarctions and microbleeds in symptomatic, as well as asymptomatic adult mutation carriers (MCs).(4;5)

Only one patient with CADASIL and a homozygous *NOTCH3* mutation has been reported to date.(6) This patient's phenotype was at the severe end of the clinical spectrum. Since CADASIL is an autosomal dominant disease, the question remains whether the disease is aggravated by a homozygous mutation.

To answer this question, we studied a Dutch female patient with a homozygous *NOTCH3* mutation and compared the clinical findings to a brother with a heterozygous mutation and a sister without a mutation.

METHODS

The female patient with the homozygous *NOTCH3* mutation is 65 years old. Her heterozygous brother is 67 years old and her sister without the mutation is 60 years old. A full clinical history was taken. An MRI examination and Neuropsychological testing were performed on the same day.

Mutation analysis

NOTCH3 mutation scanning was done by direct sequence analysis, according to previously described techniques.(7) For sequencing of exon 11 forward primer N3ex11F1 (5'-ATTGGTCCGAGGCCTCACTT) and reverse primer N3ex11R2 (5'-CCATTCCCAACCCCTCTGTG) were used. However, preferential amplification of the mutant allele can occur due to the presence of unknown polymorphisms in the primer binding site. To exclude this and to prove homozygosity of the *NOTCH3* mutation in our index case, sequencing of exon 11 was repeated with another set of primers (N3ex11F2 (5'-TGCCTGTGCTCCTGGCTACA) and N3ex12R1 (5'-TCTCATGGCAGCCACTTGCC)). We used Multiplex Ligation-dependent Probe Amplification (MLPA) to rule out that one of the *NOTCH3* alleles was deleted. Probes were designed for exon 3, 4, 9, and 11of the *NOTCH3* gene and MLPA was performed as previously described.(8) As a control probe KIAA0056 was used.

Neuropsychological Testing

The three siblings followed a standardised neuropsychological test battery, lasting three hours. Details regarding administration, scoring and clinical value of the administered neuropsychological tests have been extensively described by Spreen and Strauss.(9) Global cognitive functioning was assessed using the Groninger Intelligence Test (GIT)(10) and Cambridge Cognitive Examination (CAMCOG),(11) which taps subscores for specific cognitive functions (orientation, attention, memory, language, praxis, gnosis, calculation, abstract thinking) and incorporates the Mini Mental State Examination (MMSE).(12) Memory was additionally evaluated using the Wechsler Memory Scale (WMS).(13) For testing of executive function we used the Trail Making Test B,(14) the color-interference section of the Stroop Colour and Word test,(15) and the Symbol Digit Modalities Test (SDMT).(16)

Magnetic Resonance Imaging

Image acquisition. A uniform MRI protocol was performed on a 3.0T MR system (Philips Medical Systems, Best, The Netherlands). Conventional T1-weighted turbo field echo images (120 slices, slice thickness 1.2 mm, no slice gap, TR/TE 9.8 / 4.6 ms, matrix 192x192, and a field of view (FOV) 224x224 mm), T2-weighted spin echo images (40 slices, slice thickness 3.6 mm, no slice gap, TR/TE 3953/80 ms, matrix 448x448, FOV 224x224 mm), and Fluid Attenuated Inversion Recovery (FLAIR) images (40 slices, slice thickness 3.6 mm, no slice gap, TR/TE 10000/120 ms, inversion time 2800 ms, matrix 224x224, FOV 224x224 mm) were obtained. To specifically detect cerebral microbleeds, T2*-weighted fast field echo imaging was performed (20 slices, slice thickness 4.0 mm with an interslice gap of 1.0 mm, TR/TE 716/16 ms, matrix 256 x 256, FOV 230x230 mm). All images were performed in the axial plane parallel to the inferior border of the genu and splenium of the corpus callosum.

In order to detect ultrastructural brain tissue changes, we measured apparent diffusion coefficient (ADC) and fractional anisotropy (FA) values using diffusion tensor imaging (DTI). A diffusion-weighted single-shot EPI sequence was used, with a b-value of 750 seconds / mm2 (40 slices, slice thickness 3.6 mm, no interslice gap, TR/TE 6324/48 ms, matrix 112 x 112, FOV 224x224 mm). Diffusion encoding gradients were applied in six noncollinear directions and subsequent ADC and FA maps were calculated. ADC and FA values were determined in a region of interest (ROI) in the centrum semi-ovale of a cerebral hemisphere. The ROI size was 30 x 15 x 10 mm, located in white matter without apparent WMHs.

In order to evaluate neuronal and glial metabolism we performed single voxel MR Spectroscopy (1H-MRS) in the centrum semi-ovale of a cerebral hemisphere located in the same region as the ROI used for DTI. TR/TE 2000/38 ms. Voxel size 30 x 15 x 10 mm. Metabolite analysis was performed for N-acetylaspartate (NAA), a marker for neuronal integrity; Choline (Cho), Glutamate/Glutamine(Glx), Myo-Inositol (MI) and Creatine (Cr). Ratios were expressed rela-

tive to Creatine. Lactate (Lac), which indicates anaerobic glycolysis(17), was scored as present or absent. Ratio calculations were performed in the time domain with software delivered by the manufacturer.

White matter hyperintensities (WMHs), lacunar infarcts and microbleeds were defined as previously described.(18) WMH volume was measured using semi-automated segmentation software(19). Lacunar infarcts and microbleeds were counted manually.

RESULTS

Clinical History

Patient 1

This female patient is 65 years old. She has a clinical history of migraine with aura since the age of 14. When she was 42 years old she had an episode of confusion and loss of concentration. At the age of 64 she had a minor stroke with right sided hemiparesis and sensory loss, after which the clinical diagnosis CADASIL was made on the basis of her MRI. Apart from a slight clumsiness when walking, she reports neither neurological nor cognitive complaints. *NOTCH3* mutation scanning revealed a homozygous missense mutation of the *NOTCH3* gene in exon 11: c.C1732T resulting in a p.Arg578Cys substitution.

Patient 2

Her brother is 67 years old and recently had his first TIA at age 67. He reported slight memory loss and no other neurological complaints. He had a myocardial infarction at age 58. DNA analysis showed a heterozygous p.Arg578Cys mutation in exon 11 of the *NOTCH3* gene.

Patient 3

Her sister is 60 years old. She has a history of migraine, but proved not to have a *NOTCH3* mutation.

Family history

The parents were said not to be consanguineous, but they were born in the same vicinity (two dutch villages 15 km apart). The father died at age 67 from a myocardial infarction. He had migraine and episodes of concentration loss. The mother had migraine and died at age 53 from a cerebral stroke.

Neuropsychological Testing

The neuropsychological testing results are shown in table 1. The homozygous patient as well as her brother and sister have an above average intelligence. Memory scores were above average for all siblings. The scores for executive function of the homozygous patient (Stroop interference, Trail Making Test B, Symbol Digit Modalities Test) were slightly lower than those of her siblings. Overall, no cognitive deficits were observed.

Table 1: Neuropsychological testing results

	Patient 1 (Homozygous)	Patient 2 (Heterozygous)	Patient 3 (Wild type)
Global cognitive function			
- GIT IQ	113	113	114
- CAMCOG	96/106	99/106	95/106
- MMSE	29/30	27/30	29/30
Memory			
- WMS-MQ	122	124	120
Executive function			
- Stroop Interference Factor	95	63	24
- Trails B speed (sec)	109	94	70
- Trails B errors	1 error	0 errors	1 error
- SDMT	32	34	61
Overall Clinical Impression	No cognitive impairment	No cognitive impairment	No cognitive impairment

GIT-IQ, Groninger Intelligence Test IQ; CAMCOG, Cambridge Cognitive Examination; WMS-MQ, Wechsler Memory Scale Memory Quotient; SDMT, Symbol Digit Modalities Test.

MRI

Patient 1 (homozygous mutation)

MRI (figure 1, table 2) showed WMHs in the typical CADASIL locations: pons, anterior temporal poles, internal and external capsules. Periventricular bands of WMH were seen as well as deep WMHs, which were partly punctate and partly confluent with the periventricular bands. The volume of WMH was 63cc. A total of 5 lacunar infarcts were seen: three infarcts with diameters between 3 and 4 mm in the right centrum semi-ovale, and two infarcts with diameter 5 mm in the left centrum semi-ovale and in a periventricular location. Twenty-one microbleeds were counted.

Patient 2 (heterozygous mutation)

MRI (figure 1, table 2) showed WMHs in the peri-ventricular and deep white matter and in the internal capsules, external capsules and pons. No WMHs were seen in the anterior temporal lobe. The volume of WMH was 44cc. No lacunar infarcts or microbleeds were seen.

Figure 1. Fluid attenuated inversion recovery (FLAIR) MRI of the homozygous patient (top) and her heterozygous brother (bottom)

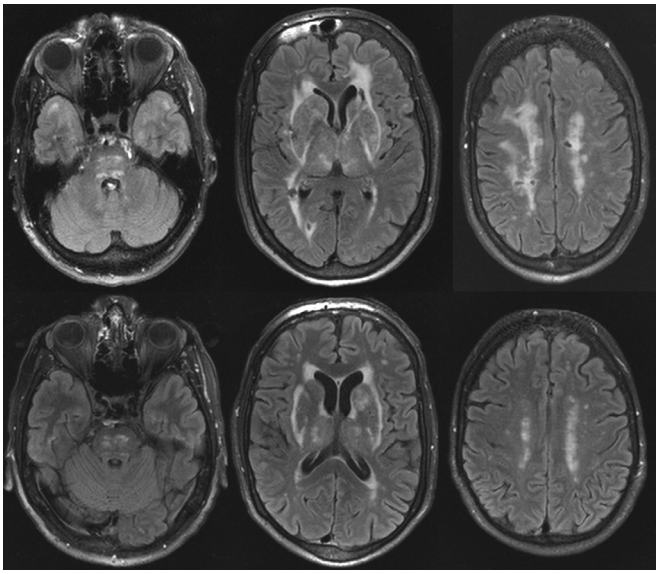

Table 2: Conventional MRI results

	Patient 1 (Homozygous)	Patient 2 (Heterozygous)	Patient 3 (Wild type)
WMH volume (cc)	63	44	0.6
Infarct count	5	0	0
Microbleed count	21	0	0

Patient 3 (no mutation)

The MRI scan of the sister without a *NOTCH3* mutation showed no infarcts or microbleeds. Small punctate WMHs (0.6cc) were seen in a pattern consistent with normal aging.

MR Spectroscopy and DTI

The results of the MR Spectroscopy are shown in table 3 and figure 2. A lactate peak was only identified in the spectrum of the homozygous patient. NAA/Cr ratios were equal in all 3 sibs. The results of the DTI are shown in table 4. The ADC and FA values of the homozygous patient were comparable to that of the heterozygous patient.

Discussion

We describe a patient who is homozygous for a known mutation in the *NOTCH3* gene (p.Arg578Cys),(1) and has a similar clinical phenotype to her heterozygous brother, both being at the very mild end of the clinical spectrum. It is still not clear whether there is a genotype phenotype correlation for disease severity in CADASIL, or which modifying factors may play a role, apart from a relatively small role of some vascular risk factors.(20;21)

Figure 2. MR Spectroscopy results

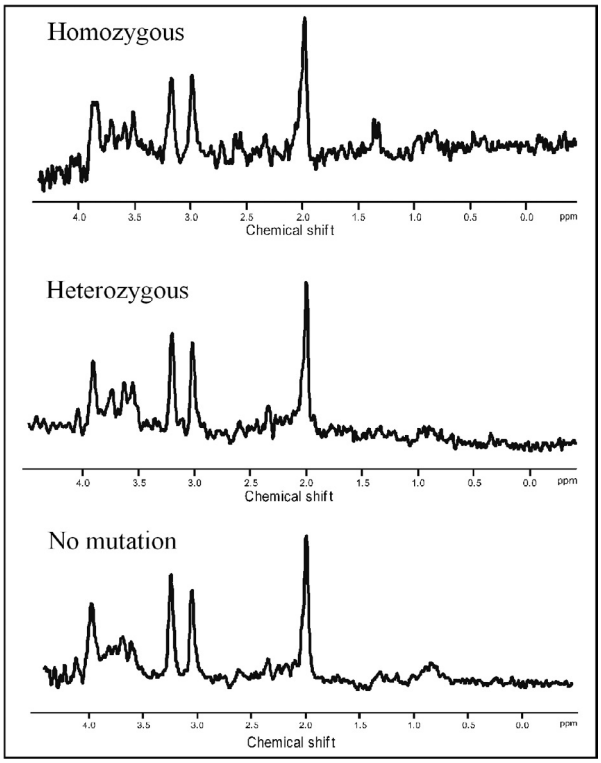

Table 3. MR Spectroscopy results

	Patient 1 (Homozygous)	Patient 2 (Heterozygous)	Patient 3 (Wild type)
Lactate	+	-	-
NAA	1.95	1.98	1.95
Choline	1.07	1.16	1.23
Myoinositol	0.49	0.68	0.27
Glx	0.49	0.33	0.49

Table 4. DTI results

	Patient 1 (Homozygous)	Patient 2 (Heterozygous)	Patient 3 (Wild type)
ADC (10^{-3} mm^2 / sec)	0.809	0.822	0.757
FA	0.35	0.40	0.35

Our patient is the second to be described with a homozygous *NOTCH3* mutation, and has a very mild phenotype whereas the previously described patient was at the severe end of the spectrum.(6) Furthermore we were able to show that the clinical phenotype in our patient did not significantly differ from that of her heterozygous brother. As in Huntington's disease, this makes CADASIL one of the rare human diseases where homozygotes are known to exist and to be phenotypically identical to heterozygotes.(22)

The homozygous patient did have more severe MRI and MRS abnormalities than her heterozygous brother. We cannot rule out that this difference is attributable to the homozygosity of the *NOTCH3* mutation. However, it should be noted that there is a large individual variance of MRI abnormalities in CADASIL patients, even within the same family,(23) and that the MRI abnormalities of this homozygous patient still fall well within the normal spectrum of MRI abnormalities for her age. The lactate peak, as found in the homozygous patient, is suggestive of recent or chronic ischemia. However, DTI testing did not confirm the presence of ischemia. FA and ADC values, measured in the same part of white matter as the MRS were normal and compatible to the value in her heterozygous brother. The normal NAA/Cr ratios in all three sibs indicate normal axonal integrity. NAA has been found to correlate most with cognitive function in various MRS studies in the general population(24). This can explain the good neuropsychological testing results in our two CADASIL patients.

From a pathophysiological perspective, our findings oppose the theory that *NOTCH3* mutations in CADASIL lead to loss-of-function.(25) In the case of a loss-of-function, one would expect that a homozygous mutation would lead to a more severe clinical phenotype, or even a lethal phenotype, because of the complete loss of NOTCH3 expression. Our findings also oppose the theory that CADASIL is caused by a dominant negative effect of *NOTCH3* mutations,(26) as dominant negative mutations require the presence of a normal allele for the disease phenotype to occur. Remaining possibilities for the pathogenetic mechanism are thus a toxic effect due to the accumulation of the mutated NOTCH3 protein or other gain of function effects.

In conclusion, the mild clinical phenotype of this homozygous CADASIL patient supports the theory that CADASIL is a classical autosomal dominant disease(6) and that patients with a homozygous or heterozygous *NOTCH3* mutation are clinically indistinguishable.

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