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CADASIL MUTATIONS INCREASE SUSCEPTIBILITY TO SPREADING DEPRESSION

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Abstract

Migraine with aura is often the first manifestation of CADASIL syndrome, a disorder caused by *NOTCH3* gene mutations expressed predominantly in vascular smooth muscle. Here, we report that cortical spreading depression, the electrophysiological substrate of migraine aura, is enhanced in mice expressing a vascular Notch 3 CADASIL mutation (R90C) or a Notch 3 knockout mutation. The phenotype was stronger in Notch 3 knockout mice implicating both loss of function and neomorphic mutations in its pathogenesis. Our results link vascular smooth muscle Notch 3 mutations to enhanced spreading depression susceptibility, implicating the neurovascular unit in the development of migraine aura.

Keywords

spreading depression; vasculopathy; migraine aura

INTRODUCTION

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy syndrome (CADASIL) is a systemic non-amyloid, non-atherosclerotic vasculopathy, with characteristic subcortical lacunar infarcts and white matter lesions. CADASIL is the most common monogenic inherited form of adult-onset stroke and vascular dementia, linked to mutations in Notch 3 expressed predominantly in vascular smooth muscle cells¹. The incidence of migraine with aura, typically the first disease symptom, is 5-times greater compared to the general population^{1–3}. Classical visual and sensory auras are most common in CADASIL, although 50% of the patients also experience atypical attacks with basilar, hemiplegic or prolonged aura, and even coma^{4, 5}. The mechanisms of this association are unknown. Among more than a hundred and fifty CADASIL mutations identified to date, the Notch 3 R90C mutation produces a strong phenotype in humans with early onset of disease symptoms^{6, 7}.

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Animal experiments as well as human imaging studies suggest that migraine aura is caused by cortical spreading depression (CSD), an intense depolarization of neuronal and glial membranes slowly propagating by way of gray matter contiguity. Evoked when extracellular K^+ concentrations ($[K^+]_e$) exceed a critical threshold, CSD is associated with massive K^+ and glutamate efflux, depolarizing adjacent neurons and glia to facilitate its spread. *Neuronal* mutations in $Ca_v2.1$ channels causing familial hemiplegic migraine type 1 augment CSD susceptibility as a mechanism for the severe migraine with aura phenotype in this syndrome⁸. Using transgenic mice expressing the human archetypal CADASIL mutation Notch 3 R90C that exhibit cerebrovascular dysfunction and most of the arterial pathological hallmarks of CADASIL, we now show that a CADASIL-associated *vascular* Notch 3 mutation also augments susceptibility to CSD, implicating CSD susceptibility as one common determinant of migraine with aura phenotype in human syndromes.

METHODS

Experimental procedures strictly followed institutional guidelines for animal care and use for research purposes, and were approved by the institutional review committee. A total of 84 mice were used in this study. Experimental groups and the numbers of mice in each group are shown in Table 1. Male and female (6–7 mo) wild type (Notch3^{WT}) and transgenic mice expressing human CADASIL mutation Arg90Cys under the control of SM22 α promoter (TgNotch3^{R90C}), were studied⁹. TgNotch3^{R90C} mice overexpress mutant Notch 3, specifically in arterial smooth muscle cells, on a wild type Notch 3 background (i.e., normal endogenous expression of wild type mouse Notch 3); therefore, we compared TgNotch3^{R90C} to transgenic mice overexpressing the human wild type Notch 3 (TgNotch3^{WT}; n=5) in addition to non-transgenic wild type controls (Notch3^{WT}). We did not observe a difference between Notch3^{WT} and TgNotch3^{WT} in any parameter, and therefore pooled the data from these two groups into a single wild type (WT) group. We also studied adult (5 mo) male Notch 3 knockout mice (Notch3^{-/-})¹⁰ and compared them to their wild type littermates (Notch3^{WT}). Although Notch3^{-/-} mice do not carry a CADASIL mutation *per se*, they model CADASIL mutations located in the ligand-binding domain that have been shown to abolish Notch 3 receptor function^{1, 7, 11}.

To address the importance of brain perfusion, we tested the impact of chronic cerebral hypoperfusion on CSD susceptibility in WT mice after bilateral common carotid stenosis (steel coils 0.18 mm internal diameter, n=6), as described previously¹². CSD susceptibility was tested 9 $\pm$ 1 weeks later, and compared to age-matched sham-operated controls (n=5).

The femoral artery was catheterized, and the trachea was intubated for mechanical ventilation under isoflurane anesthesia (2.5% induction, 1% maintenance, in 70% N₂O/30% O₂). Arterial blood gases and pH were measured every 20 min and maintained within normal limits by adjusting ventilation. Systemic physiological parameters did not differ among groups (Table 1). For analysis of CSD susceptibility, three burr holes were drilled at the coordinates described previously⁸. Two glass capillary microelectrodes were placed to record extracellular steady (DC) potential and electrocorticogram. On each hemisphere, first the electrical CSD threshold was determined by escalating intensity cathodal square pulses (10–8000 μ C) via a bipolar electrode placed on the occipital cortex, and then a 1 mm cotton ball soaked in 300 mM KCl was topically applied onto occipital cortex for 1 hour and the frequency of evoked CSDs was recorded. The amplitude, propagation speed, and duration at half-amplitude of the first CSD in each hemisphere were also measured. The data obtained from the two hemispheres were averaged to serve as a single data point for each animal. In separate experiments using TgNotch3^{R90C} mice and WT controls (n=6 each), we measured and compared blood flow changes during KCl-induced CSD (300 mM) by laser speckle flowmetry, as described previously¹³.

All experiments were done in a blinded fashion. Data were analyzed with SPSS (version 11.0). Using a general linear model of covariance analysis (ANACOVA), we tested for an effect of the independent variables genotype and age on the dependent variables cortical SD threshold, frequency and propagation speed. Other electrophysiological measures of CSD and systemic physiological data were compared among groups using one-way ANOVA. Blood flow changes during CSD were compared between groups by two-way ANOVA for repeated measures. $P < 0.05$ was considered statistically significant.

RESULTS

Direct cortical cathodal stimulation with stepwise escalating intensities triggered a CSD in all mice. The electrical threshold for CSD was approximately ten-fold lower in TgNotch3^{R90C} compared to WT mice (Figure 1A and B). Female TgNotch3^{R90C} tended to exhibit a lower CSD threshold compared to males (25 [21–55] vs. 55 [53–55] μ C; $p = 0.066$). Continuous epidural KCl application evoked repetitive CSDs in all mice. Consistent with increased CSD susceptibility, TgNotch3^{R90C} mice developed approximately 40% more CSDs during KCl application (Figure 1A and B). Propagation speed of CSD was also higher in TgNotch3^{R90C} mice compared to WT (Figure 1B); however, CSD duration and amplitude did not differ among groups (Table 1). Moreover, Notch3^{-/-} mice developed even higher numbers of CSDs in response to KCl (15 $\pm$ 2 vs. 9 $\pm$ 1 CSDs/h, in Notch3^{-/-} and WT littermates; $p < 0.001$).

CADASIL patients as well as mouse mutants develop chronic vascular dysfunction and cerebral hypoperfusion^{14, 15}. To test whether chronic hypoperfusion might explain enhanced CSD susceptibility, we induced bilateral common carotid stenosis in WT mice. However, we found that both the frequency of KCl-induced CSDs and their propagation speed were lower compared to sham controls (6.0 $\pm$ 1.4 vs. 9.2 $\pm$ 1.8 CSDs/h, 1.8 $\pm$ 0.5 vs. 2.5 $\pm$ 0.2 mm/min, $n = 6$ and 5, respectively; $p < 0.05$; Supplemental Figure 1). Moreover, setting another contrast with the TgNotch3^{R90C} phenotype, CSD durations tended to be prolonged in the stenosis group probably reflecting reduced cerebral perfusion pressure¹⁶ (Table 1). To test an alternative hypothesis that enhanced CSD susceptibility might be caused by altered vascular response to CSD, we measured CSD-induced blood flow changes using laser-speckle flowmetry and did not find a significant difference between TgNotch3^{R90C} and WT mice ($n = 6$ each; Supplemental Figure 2).

DISCUSSION

Genetic and epidemiological evidence suggests a strong association between migraine with aura and adult-onset genetic vasculopathies, such as CADASIL^{1, 17}. Using two independent but complementary techniques (electrical and KCl stimulation), we found that two different Notch 3 mutations (TgNotch3^{R90C} and Notch3^{-/-}) enhance cortical susceptibility to spreading depression as a potential mechanism for this clinical association. Enhanced CSD susceptibility in CADASIL may also explain more frequent and severe aura features (e.g., coma), similar to familial hemiplegic migraine type 1 associated with mutations in the neuronal Ca_v2.1 channels⁸.

Enhanced CSD susceptibility in both the Notch3^{-/-} and the TgNotch3^{R90C} appears paradoxical, because Notch3^{-/-} mutation is a loss-of-function, whereas Notch3^{R90C} is probably neomorphic as it retains Notch 3 function and is expressed on a wild type Notch 3 background¹⁸. However, consistent with our data in mutant mice, both neomorphic and loss-of-function CADASIL mutations are known to be associated with a migraine phenotype in affected individuals^{1, 11}.

Cerebrovascular dysfunction is a common phenotype in both mouse mutants as well as in CADASIL patients regardless of Notch 3 gain- or loss-of-function^{10, 15, 19–22}, implicating vascular mechanisms for the enhanced CSD susceptibility phenotype in Notch 3 mutants. TgNotch3^{R90C} mice do develop vascular changes characteristic of CADASIL, including disruption of smooth muscle anchorage to adjacent extracellular matrix and cells, cytoskeletal changes, and later smooth muscle degeneration⁹. Even before the morphological changes become conspicuous, TgNotch3^{R90C} mice display abnormal myogenic and flow-mediated vascular responses, impaired autoregulation and diminished hypercapnic hyperemia^{19, 20}. Further supporting a vascular link, mutations in TREX1, another vascular locus linked to the retinal vasculopathy with cerebral leukodystrophy syndrome, are also frequently associated with a migraine phenotype²³.

The mechanisms linking vascular dysfunction to enhanced CSD susceptibility, however, are not clear. Disturbances in the microcirculation caused by platelet dysfunction, inflammation, microemboli and intermittent hypoperfusion have been implicated.²⁴ It has been speculated that ischemic neuronal injury could facilitate the occurrence of CSD and migraine attacks in CADASIL²⁵. However, migraine with aura is often the first symptom in CADASIL and typically precedes ischemic events by more than a decade. Moreover, migraine attacks gradually recede at later stages of disease when ischemic injury manifests¹⁷. One possible mechanism is that blood flow dysregulation in CADASIL brains may limit the ability of cerebral vasculature to maintain energy and ionic homeostasis during intense brain activation, facilitating $[K^+]_e$ to rise above the CSD threshold. However, in separate experiments we found that chronic forebrain hypoperfusion alone, induced by bilateral common carotid artery stenosis, did not enhance KCl-induced CSD susceptibility, and that CSD-induced blood flow changes did not differ between WT and CADASIL mutants, both arguing against a primary vascular mechanism for enhanced CSD susceptibility. Additional mechanisms are therefore needed to explain enhanced CSD susceptibility in CADASIL syndrome.

Importantly, whether Notch 3 CADASIL mutations impact other cell types in the neurovascular unit has not been investigated in detail. For example, astrocyte end feet are in close proximity to cerebral blood vessels, and abnormal Notch 3 signaling may disrupt normal astrocyte-smooth muscle communication. Indeed, impaired functional neurovascular coupling was recently reported in a CADASIL mouse model expressing the Notch 3 R169C mutation¹⁴. Alternatively, Notch 3 mutations expressed in neural progenitor cells and transiently in newly born neurons may lead to enhanced CSD susceptibility phenotype later in life.^{2, 10, 17}

In summary, we found that Notch 3 mutations modulate cortical susceptibility to spreading depression, the electrophysiological substrate of migraine aura. These data are the first to link a monogenic vasculopathy to a spreading depression phenotype, and provide an explanation for the frequent and severe migraine with aura phenotype in CADASIL patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Increased CSD susceptibility in TgNotch3^{R90C} mutant mice

A) Left panel: Representative tracings showing that the electrical threshold for CSD induction was significantly lower in TgNotch3^{R90C} compared to WT mice. Stepwise escalating cathodal stimulation was used to determine the CSD threshold. **Right panel:** Representative 30 minute tracings showing that TgNotch3^{R90C} mutant mice developed significantly higher number of CSDs compared to WT during continuous topical KCl (300mM) application. The. Calibration bars: 10 mV, 5 min.

B) Left panel: Electrical threshold for CSD induction in WT and TgNotch3^{R90C} mutant mice. Each data point represents the threshold for one animal (square, male; circle, female). Medians and interquartile ranges are also shown. Please note the logarithmic ordinate scale. *, p<0.001 vs. WT. **Right upper panel:** The frequency of CSDs triggered during 60 min topical KCl application in WT and TgNotch3^{R90C} mutant mice. Topical continuous KCl application (300 mM) induced repetitive CSDs with higher frequency in TgR90C than in WT. Data are mean ± standard deviation. *p<0.001 vs. WT. **Right lower panel:** CSD propagation speed was faster in TgNotch3^{R90C} mutant mice compared to WT in both males and females (*p<0.05 vs. WT), although there was a trend towards even faster propagation speeds in female vs. male TgNotch3^{R90C} (p=0.07).

Table 1

Electrophysiological measures of CSD, and systemic physiological parameters.

Genotype/ Experimental group	Gender	N	Age (mo)	Electrophysiology			Systemic physiology			
				CSD Duration (sec)	CSD Amplitude (mV)	BP (mmHg)	pH	pCO ₂ (mmHg)	pO ₂ (mmHg)	
WT	male	12	7±1	43±12	22±2	87±3	7.39±0.02	31±3	133±18	
TgNotch3 ^{fl90C}	male	7	7±0	42±12	19±3	83±7	7.39±0.03	32±2	128±15	
WT	female	17	6±1	32±8	19±4	86±5	7.41±0.02	29±2	136±17	
TgNotch3 ^{fl90C}	female	13	6±1	33±6	18±2	86±6	7.40±0.03	31±3	136±25	
WT	male	4	5±0	54±20	30±2	76±6	7.36±0.06	32±4	151±20	
Notch3 ^{-/-}	male	8	5±0	30±4	31±3	76±8	7.39±0.04	33±2	147±22	
WT sham	male	5	4±1	40±8	20±5	82±9	7.40±0.03	30±3	164±22	
WT carotid stenosis	male	6	6±1	110±131	16±5	81±9	7.37±0.04	32±2	130±18	

Values are mean ± standard deviation. The amplitude and the duration at half amplitude of only the first CSD were measured in each mouse. Systemic physiological parameters were averaged over the entire experiment. BP, mean arterial blood pressure; pH_a, p_aCO₂, p_aO₂, arterial blood gas values.