Letter to the Editor

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Letter by Rutten et al Regarding Article, "Cysteine-Sparing CADASIL Mutations in NOTCH3 Show Proaggregatory Properties In Vitro"

To the Editor:

We read with great interest the article by Wollenweber et al,[1] proposing the use of scanning for intensely fluorescent targets to determine the pathogenicity of rare *NOTCH3* variants in the context of cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). CADASIL-causing mutations characteristically lead to an uneven number of cysteine residues in the NOTCH3 ectodomain.[2] In the vast majority of patients (>95%), a clinical diagnosis of CADASIL can be readily confirmed by the detection of a cysteine altering missense mutation in one of exons 2 to 24 of *NOTCH3*. Other CADASIL-causing *NOTCH3* mutations, such as small deletions, also induce numeric cysteine changes, supporting a fundamental role for unpaired cysteines in CADASIL pathogenesis.[3] Therefore, in the absence of such a cysteine-altering mutation, CADASIL is considered unlikely. When non–cysteine-altering variants in *NOTCH3* are detected, these are generally considered polymorphisms. There are, however, rare non–cysteine-altering variants with evidence for pathogenicity. These variants do not have characteristics distinguishing them from *NOTCH3* polymorphisms. Confirming their pathogenicity, therefore, is not straightforward and requires an indisputable clinical diagnosis of CADASIL. Because CADASIL signs and symptoms are not specific, patients with a non–cysteine-altering variant need to have a skin biopsy to confirm the diagnosis. Electron microscopy shows distinct granular osmiophilic material (GOM) in the media of skin arterioles, and immunohistochemistry shows a typical granular NOTCH3 staining.[3] Because a skin biopsy is not always feasible, a reliable functional assay to determine the pathogenicity of non–cysteine-altering variants would be of significant value, not only for diagnostic purposes but also for novel insights into CADASIL pathophysiology. Wollenweber et al[1] describe such a potential assay, namely scanning for intensely fluorescent targets to analyze aggregation properties of NOTCH3 fragments harboring non–cysteine-altering variants. They conclude that this assay can reliably distinguish *NOTCH3* pathogenic variants from polymorphisms.

As a possible missed diagnosis or misdiagnosis of CADASIL has potential far-reaching consequences, a correct classification of *NOTCH3* variants is essential. However, we have some concerns about the suggestion that scanning for intensely fluorescent targets could replace skin biopsy as a more reliable diagnostic tool. We would argue that, based on the presented data, this proposition is too premature. The critical question is whether the patients described in the article truly have CADASIL. The presence of GOM in 1 patient is certainly suggestive, but not sufficient for a conclusive CADASIL diagnosis because GOM were not found in 2 other family members with the variant. Furthermore, although GOM is considered pathognomonic for CADASIL, it is probably not 100% specific. We have seen GOM in patients with cerebral small-vessel disease, in whom CADASIL was excluded by incompatible clinical signs, the lack of a *NOTCH3* mutation and negative NOTCH3 staining (unpublished). In our experience, a 2-test skin biopsy, namely NOTCH3 staining in addition to the more widely used electron microscopy, is more reliable than electron microscopy analysis alone. Clearly, if the clinical diagnosis of CADASIL is disputable, a reliable classification of a *NOTCH3* variant is impossible. To establish a clinical diagnosis of CADASIL in patients with a *NOTCH3* variant, we recommend assessment of brain MRI and patient and family history by an experienced CADASIL team, and confirmation of the diagnosis by performing a 2-test skin biopsy. Only if the biopsy shows GOM and positive NOTCH3 staining, then the diagnosis can be considered confirmed and the variant probably pathogenic.

The comments above do not detract from the fact that scanning for intensely fluorescent targets analysis may prove to be a valuable tool in the classification of *NOTCH3* variants. However, in our opinion, sensitivity and specificity should be more firmly established, before the test can be used as a diagnostic assay in CADASIL.

Disclosures

None.

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1. Wollenweber FA, Hanecker P, Bayer-Karpinska A, Malik R, Bäzner H, Moreton F, et al. Cysteine-sparing CADASIL mutations in NOTCH3 show proaggregatory properties in vitro. *Stroke*. 2015;46:786–792. doi: 10.1161/STROKEAHA.114.007472.
2. Joutel A, Vahedi K, Corpechot C, Troesch A, Chabriat H, Vayssière C, et al. Strong clustering and stereotyped nature of Notch3 mutations in CADASIL patients. *Lancet*. 1997;350:1511–1515. doi: 10.1016/S0140-6736(97)08083-5.
3. Rutten JW, Haan J, Terwindt GM, van Duinen SG, Boon EM, Lesnik Oberstein SA. Interpretation of NOTCH3 mutations in the diagnosis of CADASIL. *Expert Rev Mol Diagn*. 2014;14:593–603. doi: 10.1586/14737159.2014.922880.

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