Response to Letter Regarding Article, “Cysteine-Sparing CADASIL Mutations in NOTCH3 Show Proaggregatory Properties In Vitro”

We thank Rutten et al.1 for their comments on our work on cysteine-sparing NOTCH3 mutations in cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL).2 We fully agree that the diagnostic workup of patients with atypical mutations requires an experienced CADASIL team and needs to incorporate all available information including medical and family history, clinical and neuropsychological examination, biopsy results, neuroimaging data, and sequencing of at least all NOTCH3 exons encoding EGF repeats. In that respect, the majority of previously published reports on cysteine-sparing mutations were incomplete. Hence, the CADASIL diagnosis in some of these studies can indeed be questioned. In accord with this, our in vitro aggregation assay suggests heterogeneity among previously reported cysteine-sparing mutations. Therefore, we took great care to provide a comprehensive and high-quality workup of the patients within our family. The diagnosis CADASIL was made only after a thorough clinical, molecular, and pathological examination. Results largely met the criteria recommended by Rutten et al.3 In addition, information from genome databases such as the Exome Sequencing Project was included to estimate the population frequency of the identified D80G mutation. Variants with a frequency largely exceeding the frequency of CADASIL are unlikely to represent a disease-causing mutation. Unfortunately, immunostaining of NOTCH3 aggregates could not be performed in our study. It should, however, be noted that immunostaining is also considered to be only supportive rather than definite for diagnosing CADASIL owing to its sensitivity of ≈85% to 90%.4 In conclusion, in our view, little doubt remains that the patients presented in our study truly have CADASIL.

Interestingly, granular osmiophilic material could be detected in only 1 patient. Although granular osmiophilic material is pathognomonic for CADASIL and NOTCH3 aggregation is considered a key factor in the pathogenesis, the mechanisms of toxicity within vascular walls are poorly understood. As discussed in our article, large deposits detectable as granular osmiophilic material might merely represent an end point in the NOTCH3 aggregation process without a key role in disease pathogenesis. As demonstrated in neurodegenerative diseases such as Alzheimer disease intermediates in the aggregation process, not the end product, might represent the toxic agent.5 Thus, the development of CADASIL in the absence of detectable granular osmiophilic material is not completely inconceivable. Therefore, we feel that our in vitro single particle aggregation assay adds valuable information when categorizing novel variants with respect to their clinical significance. It is important to note that we propose our single particle aggregation assay as an additional tool to assess the pathogenicity of NOTCH3 mutations, not as replacement for a skin biopsy. We agree with Rutten et al.1 that the significance of single particle aggregation assay data can only be evaluated in combination with a thorough and high-quality diagnostic workup using the tools discussed above.

Ultimately, in the absence of a gold standard, a public database of NOTCH3 mutations (typical and atypical) collecting clinical, neuroimaging, biopsy and perhaps single particle aggregation assay information could help to further elucidate the role of cysteine-sparing mutations and facilitate diagnostic decision-making.

Disclosures

None.

Frank Arne Wollenweber, MD
Christof Haffner, PhD
Marco Duering, MD
Institute for Stroke and Dementia Research
Klinikum der Universität München, Ludwig-Maximilians-University Munich, Germany


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