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A cardinal sin when researching neuropsin/KLK8: Thou shalt validate antibodies

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We read with great interest the recent article by Herring et al. [1], who presented a highly detailed explication of potential roles for kallikrein 8 (KLK8) in Alzheimer's disease (AD); their work highlights how KLK8 inhibition attenuates AD pathology in mice [1]. They illustrated increased KLK8 protein in AD brain, progressing by Consortium to Establish a Registry for Alzheimer's Disease (CERAD) stage. But increase in KLK8 in TgCRND8 ($APP_{SWE/IND}$) mice suggests a role for increased amyloid-beta (Aβ) protein precursor (APP) levels, increased Aβ levels, and/or disrupted Aβ42/40 ratios in altering KLK8 levels. What is not clear is the sequence of the biochemical cascade. Did excess APP levels cause increase in KLK8 or vice versa? They also demonstrated behavioral and neuropathologic benefits for blockading KLK8 in situ in the transgenic mice, including reduction in Aβ plaque load, τ hyperphosphorylation, and changes in APP processing. Likewise, KLK8 blockade produced memory deficits in wild-type mice. The mechanism they proposed included both KLK8 activity on ephrin receptor B2 (EPHB2) and several other KLK8 substrates. KLK8 increase has long been known in the study of AD [2]. However, they did not show effects of KLK8 on other early memory-relevant gene products, such as cyclic adenine monophosphate response element binding protein (CREB) activity. How KLK8 relates to cholinergic pathways is also not discussed. Cholinergic dysfunction is well known to associate with AD, and in particular, muscarinic acetylcholine receptor (mAChR) agonists are potential drug candidates for treatment of AD [3].

Unfortunately, the matters were not as nicely tied up as one might wish. The report fails to sufficiently indicate the nature of immunoreactive bands detected by Western immunoblotting techniques, and their Western results form the basis of interpretation of their

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whole work. This is related to a problem that is pandemic in molecular biology (including protein) research validation. Notably, Herring et al. did not once indicate a specific molecular weight size in any of 100 depictions of Western blot bands, either in main or supplemental figures. Likewise, nowhere in the text were the specific molecular weights of bands visualized clearly mentioned. In addition, we are not aware if validation in the context of a Western blot for specificity in samples tested was done. We recognize that the article did mention some non-Western technique validation, in that its blockade antibody was specifically tested against thrombin, trypsin, tissue plasminogen activator, and urokinase plasminogen activator. But in the same sentence, there was mention of validation for nonreactivity versus “kallikrein,” which is a 15-member family that includes KLK8. Among the copious supplementary data, there was not a single full-length blot. In this day and age, given that supplemental data is now always online, perhaps we might wish for a full-length Western blot with each primary antibody, indicating the specific band counted as the intended target. These certainly could be purely technical deficiencies, but the matter of antibody validation can no longer be simply taken as a matter of course [4]. Monoclonal antibodies, such as 22C11, which recognizes a well-characterized epitope within APP in both rodents and humans [5], were discovered to cross-react with the APP superfamily member APLP2 [6]. The antibody used by Herring et al. was a polyclonal serum rather than a monoclonal, and the KLK family has within itself regions of very high homology (shared with KLK8) and similar molecular weights [7]. This is particularly of concern because both KLK7 and KLK10 are also increased in AD [8]. How much these local homologies may be reflected in cross-reactivity of specific antisera is simply not reported, so far as we know. Even presuming perfect integrity on the part of workers, we ought no longer to presume our reagents match our ideals. The International Working Group for Antibody Validation [9] is working to formulate best practices for validating antibodies used in common research applications and to provide guidelines that ensure antibody reproducibility. Currently, readers may be wondering whether it is KLK8/neuropsin performing the “sin” in AD brain or it is using invalidated antibodies in Western blotting. However, we have, to make a further salient point, indulged in another all-too-common practice. In this letter we used an imprecise common term, specifically “neuropsin”. Neuropsin does not uniquely refer to KLK8 protein. The same word can also refer to opsin 5, which is encoded by OPN5 [10]. KLK8 is a serine protease, while OPN5 is a photoreceptor protein. Both are commonly referred to as “neuropsin”, within the same research and medical fields. A great deal of historical weight may be behind such imprecise terminology, like the current Wild Western approach to antibody validation, but both carry risk of costly confusion.

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