American Journal of Pathology, Vol. 156, No. 2, February 2000

Correspondence

A Proposed BAT-26 Germline Polymorphism

To the Editor-in-Chief:

In a recent issue of *The American Journal of Pathology*, Samowitz et al[1] reported on the instability of the mono-satellite markers BAT-26 and BAT-40 in colorectal adenomas and carcinomas. The study identified a second, significantly shorter allele of BAT-26 and suggested that a germline polymorphism exists for BAT-26 in 7.7% of African-Americans and 0.08% of Caucasians. This raises the possibility that misclassification of replication error status in tumors may occur, especially in African-Americans, if based solely on analysis of tumor material.

We wish to draw attention to the possibility that methodological errors may have led to the observation of this shorter allele. In our experience[2] using the ABI 377 (PE Applied Biosystems, Warrington, UK), constitutional DNA analyzed for BAT-26 with a fluorescently labeled PCR primer occasionally gives rise to a trace suggesting the presence of a shorter allele. However, this is never reproducible. Moreover, in both of the traces obtained with BAT-26 primers on constitutional DNA shown by Samowitz et al in their Figure 2a, the fluorescence signal is less than 150 relative units. According to PE Applied Biosystems, under 150 units "the signal-to-noise ratio is too low to discriminate between sample peaks and background."[3] Furthermore, on both traces there are additional signals at around 85 length units, indicating a high background. It is unclear from the paper whether all cases with shortened alleles were confirmed using constitutional DNA extracted from lymphoblastoid cells. This, therefore, leaves open the possibility that an apparently shorter germline BAT-26 allele may have arisen from contamination with tumor tissue harboring that allele.

Unequivocal evidence for the presence of a shorter germline allele would come from detection of a homozygote. However, we acknowledge that given a heterozygote frequency of 7.7% in 65 African-Americans, there is only limited power to detect this. In the absence of unambiguous evidence, we believe there are sufficient methodological issues to draw into question the conclusion that there exists a germline BAT-26 allele that is significantly shorter than normal.

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Author's Reply:

Bradshaw et al are concerned that our novel identification of BAT-26 polymorphisms (and, presumably, the increased frequency of these polymorphisms in African-Americans) could have been the result of contamination with tumor and/or an artifact due to low signal and high background. With regard to the first concern, shortened BAT-26 alleles were identified in DNA extracted from peripheral blood lymphocytes from 6 out of the 12 individuals with germline polymorphisms listed in Table 2 or our paper.[1] This result rules out tumor contamination as the source of these novel alleles. With regard to the second concern, we feel that our published data are convincing (and both of the novel allele peaks are actually higher than the 150 relative units cited as necessary to reliably distinguish signal from background). Nevertheless, we are happy to present additional data from the study that resolve this point. Figure 1 shows BAT-26 results from peripheral blood lymphocyte DNA from two individuals listed in Table 2 of our paper.[1] A novel allele (designated B), approximately 10 basepairs smaller than full-length BAT-26 (designated A), is seen in both samples (the absolute size of alleles A and B is increased because of a 6-bp addition to the reverse primer[2]). There is very low background, and the amplitude of this shortened allele is above 1000 relative units.

We would also like to point out that a recent study published in *The American Journal of Pathology* has repro-

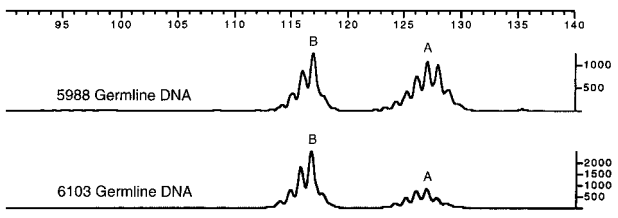

Figure 1. BAT-26 polymorphisms in two individuals listed in Table 2 of our paper.[1] A novel allele, designated B, approximately 10 bp smaller than full-length BAT-26, designated A, is present in both samples. DNA was prepared from peripheral blood lymphocytes. Size in basepairs is indicated by the scale at the top of the figure; signal amplitude is indicated by the scale on the right.

duced our finding of BAT-26 polymorphisms and the increased frequency of these polymorphisms in African-Americans.[3]

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The Role of NAC in Amyloidogenesis in Alzheimer's Disease

To the Editor-in-Chief:

In 1993, Saitoh and colleagues identified the non-Aβ component of Alzheimer's disease (AD), amyloid (NAC), as an important element of amyloid-enriched fractions in AD brains.[1] NAC is a 35-amino acid (aa) fragment derived from its 140-aa precursor, α-synuclein, which is now recognized to play a major role in Lewy body disease (LBD) pathogenesis.[2] Furthermore, immunohistochemical studies with the anti-NAC-X1 antibody revealed that NAC is closely colocalized with Aβ in the AD plaques.[1] Moreover, biochemical studies showed that NAC is extremely hydrophobic and easily forms amyloid-like fibrils under physiological conditions.[3] These results led us to the hypothesis that NAC might play an important role in Aβ aggregation and amyloidogenesis in AD.[1]

In a recent issue of *The American Journal of Pathology*, Culvenor et al[4] reported that NAC may not be associated with Aβ in the plaques. Using the antibody NAC42580 to label cortical and hippocampal sections of AD cases, the authors found no evidence of NAC immunoreactivity in the plaques. Furthermore, although the NAC42580 showed certain immunoreactivity in the urea extracts of the sodium dodecyl sulfate (SDS)-insoluble fractions in diffuse LBD and Parkinson's disease (PD) cases, there was no correlation between NAC and Aβ immunoreactivity in the same AD fractions. Based on this study and on a previous report,[5] the authors concluded that NAC may not be associated with Aβ in the plaques of AD.

Several possibilities could be considered to explain the discrepancy between our results and those reported by Culvenor et al.[4] First, differences in immunoreactivity could be due to differential properties of antibodies used in each study. NAC-X1 and NAC42580 were raised against epitopes derived from different portions of the NAC region: NAC1–9 for NAC-X1[1] and NAC15–31 for NAC42580.[4,5] It is important to note that immunoblotting analysis showed that NAC-X1 preferentially recognizes aggregated forms of the NAC molecule over the monomeric ones.[3] Furthermore, in our experience, NAC-X1 does not immunoreact with soluble α-synuclein.[3] Thus, it can be predicted that our NAC-X1 antibody may be ideal to detect aggregated NAC in the plaques of AD. In contrast, immunoblotting analysis with the NAC42580 showed immunoreactivity with soluble α-synuclein in brain tissues.[4,5] In fact, biochemical analysis of the NAC peptide by El-Agnaf et al[6] showed that the N-terminal region of NAC (NAC1–18) aggregates to form amyloid fibrils, while the C-terminal region (NAC19–35) remains soluble, suggesting that the N-terminal portion is essential for aggregation of NAC peptide.[6] Taken together, these observations might suggest that the NAC1–9 peptide could be naturally aggregated during inoculation in the rabbit, leading to the production of NAC-X1, which preferentially recognizes aggregated forms of NAC, whereas the C-terminal region of NAC may be less effective.

Second, differences in antibody immunoreactivity might depend on the methods used for tissue preparation. In this regard, the NAC-X1 antibody immunolabeled plaques in paraformaldehyde-fixed vibratome sections, but not in formalin-fixed, paraffin-embedded tissue.[1,3,7,8] In fact, both Bayer and Culvenor used archival formalin-fixed and/or paraffin-embedded tissues, rather than vibratome, for their immunohistochemical studies,[4,5] raising the possibility that fixation, solvents, and paraffin might destroy the NAC-X1 epitope. Further supporting the importance of tissue pretreatment and processing to detect the NAC epitope, we have recently shown that in vibratome sections pretreated with formic acid, the NAC-X1 antibody immunostained not only amyloid plaques and amyloid angiopathy, but also astroglial cells and granular neurons in LBD.[7]

Finally, it is possible that the NAC-X1 antibody might be cross-reacting with or recognizing protein quaternary structure. In this regard, immunoblotting analysis confirmed that both monomeric and aggregated forms of Aβ were not immunoreactive with NAC-X1.[1] Therefore, it is unlikely that NAC-X1 cross-reacts with Aβ.

In summary, we would like to argue that it is necessary to continue exploring the hypothesis that NAC plays an important role in plaque formation and AD. The essential difficulty in obtaining conclusive evidence may be due to the lack of information as to the mechanisms by which NAC is generated from its precursor, α-synuclein.[2] In this regard, the results of this study by Culvenor et al[4] are potentially interesting, because their immunoblotting analysis of brain homogenates showed that in addition to