



Published in final edited form as:

*Kidney Int.* 2016 March ; 89(3): 721–723. doi:10.1016/j.kint.2015.10.019.

## Adriamycin susceptibility among C57BL/6 substrains

Ehtesham Arif<sup>1</sup>, Ashish K. Solanki<sup>1</sup>, and Deepak Nihalani<sup>1</sup>

<sup>1</sup>Nephrology Division, Medical University of South Carolina, Charleston, South Carolina, USA

### To the Editor

The C57BL/6 mouse inbred strain is the preferred strain for generating various knockout, knock-in, and genetic glomerular disease models. A review of published literature in nephrology journals reveals that C57BL/6 and C57BL/6J have been used interchangeably, largely ignoring the fact that there are significant substrain differences within the C57BL/6 strain.<sup>1,2</sup> Thus, it is critical that the nephrology community is well aware of the genetic variations among C57BL/6 substrains<sup>3</sup> that result in varying susceptibility toward various injury-inducing agents and may have important implications for results and their interpretation. When the C57BL/6J substrain was shown to resist adriamycin-induced glomerulopathy,<sup>4,5</sup> it led to a widespread notion that the C57BL/6 strain is resistant to adriamycin.<sup>4</sup> In the present investigation, we demonstrate that unlike C57BL/6J, the C57BL/6N substrain is susceptible to adriamycin-induced glomerulopathy. Adriamycin or saline was retro-orbitally administered in 10-week-old C57BL/6J and C57BL/6N mice. Pre- and post-injection urine samples were collected and analyzed by sodium dodecylsulfate–polyacrylamide gel electrophoresis and enzyme-linked immunosorbent assay for albuminuria estimation. While C57BL/6J mice showed complete absence of albuminuria as reported previously (data not shown),<sup>2,4</sup> the C57BL/6N mice displayed significant albuminuria at 4 weeks (Figure 1a and b). Further analysis by scanning and transmission electron microscopy showed significant loss of podocyte morphology and foot process effacement that is consistent with adriamycin-induced glomerulopathy (Figure 1c and d). These results are consistent with recent genetic analysis that suggests these 2 substrains are genetically distinct and vary in their susceptibility to a number of factors.<sup>3</sup> We believe that these results should persuade investigators and reviewers to consider the substrain differences when reporting their results and during the review of manuscripts where such a difference could have significant implications.

### References

1. Hakrrouch S, Cebulla A, Schaldecker T, et al. Extensive podocyte loss triggers a rapid parietal epithelial cell response. *J Am Soc Nephrol.* 2014; 25:927–938. [PubMed: 24335975]
2. Johnstone DB, Zhang J, George B, et al. Podocyte-specific deletion of Myh9 encoding nonmuscle myosin heavy chain 2A predisposes mice to glomerulopathy. *Mol Cell Bi006Fl.* 2011; 31:2162–2170.

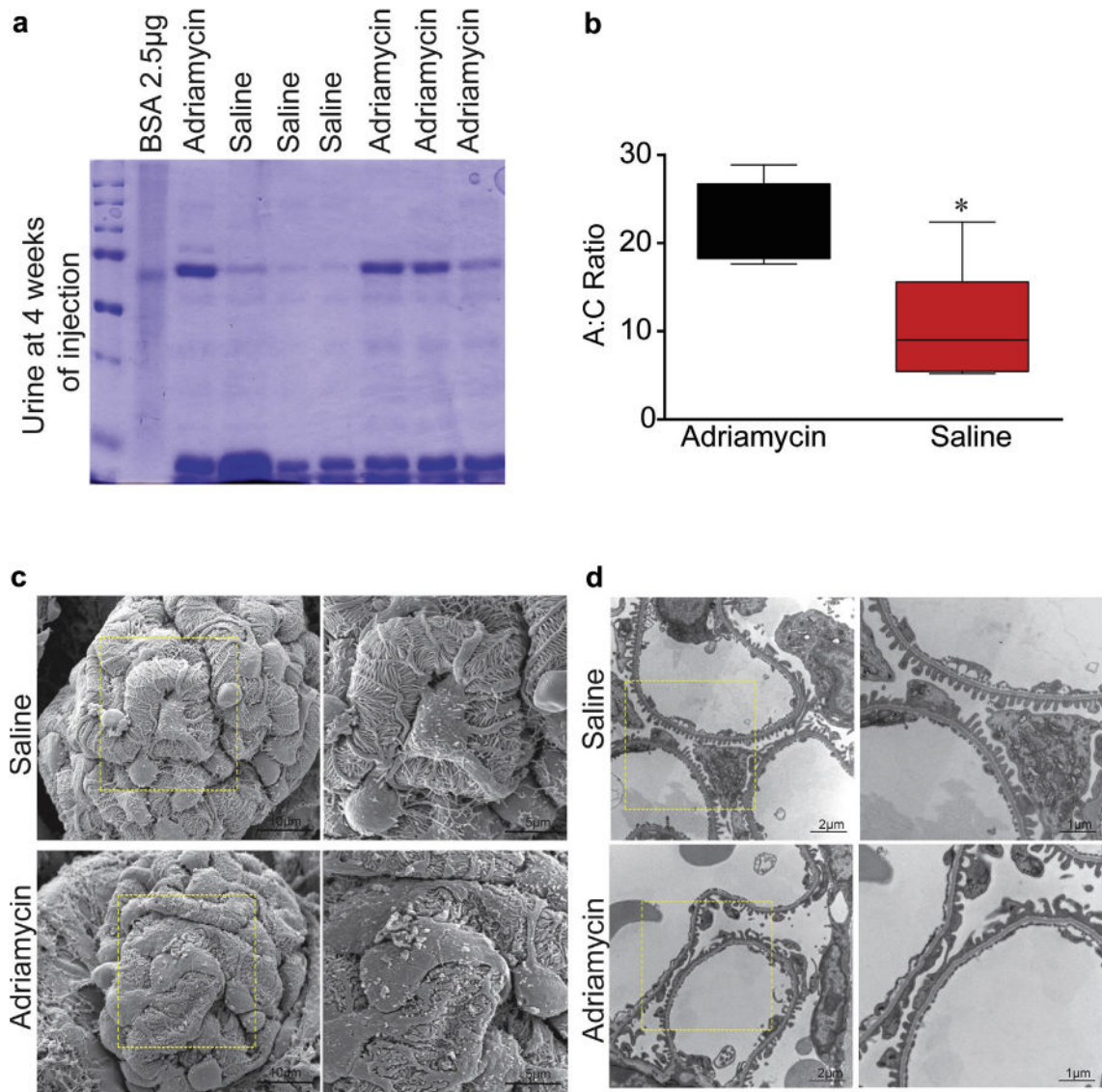
3. Simon MM, Greenaway S, White JK, et al. A comparative phenotypic and genomic analysis of C57BL/6J and C57BL/6N mouse strains. *Genome Biol.* 2013; 14:R82. [PubMed: 23902802]
4. Jeansson M, Bjorck K, Tenstad O, Haraldsson B. Adriamycin alters glomerular endothelium to induce proteinuria. *J Am Soc Nephrol.* 2009; 20:114–122. [PubMed: 19073829]
5. Simons M, Hartleben B, Huber TB. Podocyte polarity signalling. *Curr Opin Nephrol Hypertens.* 2009; 18:324–330. [PubMed: 19542980]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript



### Figure 1. C57BL/6N mice are susceptible to adriamycin-induced injury

Adriamycin or saline was retro-orbitally injected in anesthetized C57BL/6N mice ( $n = 6-8$ ) (15 mg/kg of body weight). Urine from each mouse was collected at 1-week intervals, and the 4-week urine samples were analyzed by sodium dodecylsulfate–polyacrylamide gel electrophoresis followed by Coomassie blue staining and enzyme-linked immunosorbent assay to calculate albumin-creatinine ratios. Albuminuria was noted only in adriamycin-injected mice (**a** and **b**;  $*P < 0.05$ ). Scanning and transmission electron microscopy analysis of kidney tissues from these mice further confirms podocyte damage in adriamycin-injected mice only (**c** and **d**). BSA, bovine serum albumin.