

COMMENTARY

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## The Alternatively Spliced Form “b” of the Epithelial Sodium Channel $\alpha$ subunit ( $\alpha$ ENaC): Any Prior Evidence of its Existence?

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**Abstract:** The epithelial sodium channel (ENaC) is critical in maintaining sodium balance across aldosterone-responsive epithelia. ENaC is a combined channel formed of three subunits ( $\alpha\beta\gamma$ ) with  $\alpha$  ENaC subunit being the most critical for channel functionality. In a previous report, we have demonstrated the existence and mRNA expression levels of four alternatively spliced forms of the  $\alpha$  ENaC subunit denoted by -a, -b, -c and -d in kidney cortex of Dahl S and R rats. Of the four alternatively spliced forms presently identified,  $\alpha$  ENaC-b is considered the most interesting for the following reasons: Aside from being a salt-sensitive transcript,  $\alpha$  ENaC-b mRNA expression is ~32 fold higher than  $\alpha$  ENaC wildtype in kidney cortex of Dahl rats. Additionally, the splice site used to generate  $\alpha$  ENaC-b is conserved across species. Finally,  $\alpha$  ENaC-b mRNA expression is significantly higher in salt-resistant Dahl R rats *versus* salt-sensitive Dahl S rats. As such, this commentary aims to highlight some of the previously published research articles that described the existence of an additional protein band on  $\alpha$  ENaC western blots that could account for  $\alpha$  ENaC-b in other rat species.

**Keywords:**  $\alpha$  ENaC-b, Dahl rats, salt-sensitive hypertension, Western analysis

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## Commentary

Over the past decades, ENaC has been extensively studied in multiple species because of its critical role in maintaining sodium balance across aldosterone-responsive epithelia. ENaC is twice as active in the inner medullary collecting ducts of Dahl salt-sensitive (S) *versus* salt-resistant (R) rats.<sup>1</sup> While it is tempting to speculate that ENaC mutations might be the reason behind the enhanced ENaC activity in Dahl S *versus* R rats, recently a comprehensive ENaC  $\alpha$ ,  $\beta$ , and  $\gamma$  screening study had reported the identical sequences of ENaC  $\alpha$ ,  $\beta$ , and  $\gamma$  genes in the exon-intron boundaries, 5' and 3' flanking regions as well as the complete coding regions.<sup>2</sup>

There were no reports on the RNA regulation of ENaC in Dahl S and R rat models until recently when Aoi et al reported an abnormal increase in  $\alpha$  ENaC mRNA (2.5-fold) in the kidneys of Dahl S, but not R rats ( $\alpha$  ENaC mRNA is suppressed in Dahl R rats) on high *versus* regular salt intake for 4 weeks.<sup>3,4</sup> It is worth mentioning at this point that the sequences of the forward and reverse primers used by Aoi and co-authors<sup>3,4</sup> are common among  $\alpha$  ENaC wildtype (wt),  $\alpha$  ENaC-a and -b spliced forms,<sup>5,6</sup> and the altered levels reported for  $\alpha$  ENaC should be carefully interpreted (because the primers would amplify both the major transcript and potentially the two alternatively spliced forms [-a and -b], and hence cause false elevations of full-length  $\alpha$  ENaC mRNA). Our previously reported data,<sup>6</sup> on the other hand, showed no significant changes in full-length  $\alpha$  ENaC mRNA concentration in response to salt in Dahl S and R rats, but a constitutive increase in  $\alpha$  ENaC mRNA concentration was witnessed in Dahl R *versus* S rats. As such, the reasons for ENaC functional variability in Dahl rats are poorly understood at present. A potential explanation for the differential ENaC regulation in Dahl rats is via alternative splicing of the principle  $\alpha$  ENaC subunit, which is the focus of the present commentary.

Surprisingly, until very recently alternative splicing of ENaC has not been the focus of biomedical research. In a previous report,<sup>6</sup> four alternatively spliced forms (-a, -b, -c and -d) associated with  $\alpha$  ENaC were defined in Dahl S and R rats in terms of their existence, their mRNA expression levels, and their mRNA profiles in response to high salt diet. Among the four  $\alpha$  ENaC alternatively spliced

forms,  $\alpha$  ENaC-b is the most interesting because of the following factors.  $\alpha$  ENaC-b mRNA expression levels are significantly higher in Dahl R rats with suppressed ENaC activity *versus* Dahl S rats (a sister strain with an enhanced ENaC activity).<sup>6</sup>  $\alpha$  ENaC-b is a salt-sensitive transcript whose mRNA expression levels are ~32 fold higher than  $\alpha$  ENaC wildtype.<sup>6</sup> Finally, the splice site used to generate  $\alpha$  ENaC-b is conserved across species.<sup>5,6</sup>

Overrepresentation of  $\alpha$  ENaC-b in Dahl R could be indicative of a dominant negative effect on  $\alpha$  ENaC expression/function in this model. In some instances of dominant negative effects, it is the excessive accumulation of the translated peptide that competes with full-length for activity.<sup>7</sup> It is worth mentioning that increased preference of  $\alpha$  ENaC-b mRNA and possibly protein synthesis in Dahl R rats might have been encouraged by the initial constitutive increase in full length  $\alpha$  ENaC mRNA in this strain, to allow for more  $\alpha$  ENaC-b protein formation and accumulation.

As such, we were curious to find out if other research papers did in fact highlight the existence of an additional band of the size of  $\alpha$  ENaC-b on  $\alpha$  ENaC western blots. It is worth mentioning at this point that  $\alpha$ -ENaC antibody we employed in our experiments<sup>17</sup> is directed against the N-terminus amino acid residues 46–68 [NH<sub>2</sub>-LGK GDKREEQGLGPEPSAPRQPT-COOH] of the rat ( $\alpha$  ENaC). The  $\alpha$ -ENaC antibody is supposed to recognize protein bands of apparent masses of 80–85 ( $\alpha$  ENaC) and 37 kDa (a proteolytic fragment of  $\alpha$  ENaC). Previous channel purification studies<sup>5</sup> and  $\alpha$  ENaC western blots have demonstrated extra proteins that could account for additional alternatively spliced forms that might play a role in ENaC structure/function. During our search for existing western blots for  $\alpha$  ENaC, we identified several articles that described  $\alpha$  ENaC protein expression by presenting the  $\alpha$  ENaC subunit protein band alone without showing any additional protein bands.<sup>8–12</sup> On the other hand, several other researchers reported the presence of an additional band of the size 49–60 kDa.<sup>13–15</sup> The size of  $\alpha$  ENaC-b of 53 kDa<sup>16,17</sup> could easily match the size of the existing band in the above western blots. While some authors described the additional band (s) as non specific to  $\alpha$  ENaC antibody or anonymous, other authors attributed the existence of this consistent band to the possibility of being a yet to be identified  $\alpha$  ENaC cleavage product. As such, it is essential



to confirm at this point the *in vivo* expression of  $\alpha$  ENaC-b in kidney tissues as described by our recently published hypothesis detailing the step-by-step approach to examining  $\alpha$  ENaC-b.<sup>18</sup>

Therefore, this commentary does not prove or disprove the protein expression of  $\alpha$  ENaC-b in other rat species, but rather confirms the existence of several yet to be identified protein bands related to  $\alpha$  ENaC. The presence of several bands on  $\alpha$  ENaC western blots constitutes the driving force to pursue  $\alpha$  ENaC-b protein expression *in vivo versus* if none of the above yet to be determined protein bands were present.

## Disclosure

This manuscript has been read and approved by the author. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The author and peer reviewers of this paper report no conflicts of interest. The author confirms that they have permission to reproduce any copyrighted material.

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