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Invited review

# Genetic exploration of the role of acid-sensing ion channels

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ABSTRACT

Advanced gene targeting technology and related tools in mice have been incorporated into studies of acid-sensing ion channels (ASICs). A single ASIC subtype can be knocked out specifically and screened thoroughly for expression in the nervous system at the cellular level. Mapping studies have further shed light on the initiation and identification of related behavioral phenotypes. Here we review studies involving genetically engineered mouse models used to investigate the physiological function of individual ASIC subtypes: ASIC1 (and ASIC1a), ASIC2, ASIC3 and ASIC4. We discuss the detailed expression studies and significant phenotypes revealed with gene knockout for most known *Asic* subtypes. Each strategy designed to manipulate mouse genetics has advantages and disadvantages. We discuss the limitations of these *Asic*-knockout models and propose future directions to solve the genetic issues.

This article is part of a Special Issue entitled 'ASIC Channels'.



## 1. Introduction

Named for the capacity to depolarize neurons on proton stimulation, acid-sensing ion channels (ASICs) have drawn much attention among neuroscientists in the past 2 decades (Waldmann et al., 1997). Belonging to the degenerin/epithelial sodium channel (DEG/ENaC) family, ASICs have a common protein structure characterized by 2 transmembrane domains, 1 extracellular loop and intracellular N- and C-terminal domains. Under physiological or pathological conditions, environmental acidification occurs during vigorous synaptic transmission, local ischemia, energy consumption and inflammatory tissue damage. Therefore, ASICs may play important roles in these functions. However, the required sensitivity, current evocation, modulators and even downstream effectors for a given acid strike differ greatly among tissues, nuclei, and cell types in the nervous system. Life solves the problem in several ways. First, various functional subunits of ASICs have been conserved during evolution: in rodents, 4 different genes, including *Accn1 (Asic2)*, *Accn2 (Asic1)*, *Accn3 (Asic3)* and *Accn4 (Asic4)* encode ASICs (Baron et al., 2013). *Accn5* encodes a related channel, BLINaC/BASIC that is however clearly different from ASICs, although it has been renamed ASIC5 by some authors (Schaefer et al., 2000; Wiemuth et al., 2013). Second, transcript variants are multiplied via alternative splicing among exon compositions: in the mRNA database from the UCSC (University of California, Santa Cruz) and the VEGA (vertebrate genome annotation) genome browser, at least 2, 3, 2, 2, and 2 spliced variants have been reported from mouse *Accn1*, *Accn2*, *Accn3*, *Accn4*, and *Accn5*, respectively (Table 1). Because exon splicing involves different 5′ untranslated regions (5′-UTRs) (exon 1) and 3′-UTRs, the information for the cell-type-–specific promoter is therefore wired into different ASIC isoforms. Third, the channel composition diverges via subunit multimerization: at the protein level, ASICs function as a trimer, with either homotrimeric or heterotrimeric channels.

The major members of the ASIC family investigated include ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3 and ASIC4. More ASIC members or splice variants remain to be identified. Also, the exact number, composition, and cellular expression of these known or unknown ASIC subunits require future studies for validation. In parallel with the advances in gene targeting technology in mouse, many of the *Accn* genes have been knocked out and several phenotypes have been addressed. In this review, we focus on research into these *Asic* gene-targeting mice from year 2000–2014. The literatures addressing ASICs are increased dramatically over the past

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0028-3908/

**Table 1**
A summary of different ASIC subtypes in mice with their corresponding exon number in mRNA and amino acid number in translated protein.

| Gene | ASIC subtype | Exon | Amino acid | Accession no. |
|---|---|---|---|---|
| *Accn2* | ASIC1a | 12 | 526 | BC067025 |
| | ASIC1b | 10 | 559 | AB208022 |
| *Accn1* | ASIC2a | 10 | 512 | AF348465 |
| | ASIC2b | 10 | 563 | Y14634 |
| *Accn3* | ASIC3(a) | 11 | 530 | BC054460 |
| | ASIC3(b) | 10 | 494 | AY261387 |
| *Accn4* | ASIC4(a) | 10 | 539 | BC055772 |
| | ASIC4(b) | 9 | 520 | BC046481 |
| *Accn5*[a] | ASIC5(a) | 10 | 495 | BC140300 |
| | ASIC5(b) | 9 | 453 | AK038321 |

[a] *Accn5* encodes a channel that is clearly different from other ASICs and is identified as BLINaC/BASIC and has been renamed ASIC5 by some authors.

two decades, especially for phenotyping studies of ASIC1, 2, and 3 (Fig. 1). We summarize, compare and discuss phenotyping results from these studies. This review covers different strategies and technical concerns in generating *Asic*-knockout models, expression studies of ASICs with knockout mice, phenotypes of *Asic*-knockout mice and future perspectives for ASIC studies.

## 2. Strategies and technical concerns in generating *Asic* knockouts

The existing mutant mouse models for the ASIC family and their individual gene-targeting strategies are summarized in Fig. 2. Three gene-targeted mouse models have been reported for *Asic1* (Wemmie et al., 2002; Staniland and McMahon, 2009; Wu et al., 2013), 2 for *Asic2* (Price et al., 2000; Ettaiche et al., 2004), 6 for *Asic3* (Chen et al., 2002; Xie et al., 2002; Drew et al., 2004; Wultsch et al., 2008; Lin and Chen, 2011), 1 for *Asic4* (Lin and Chen, 2012), and 1 for *Asic5* (Boiko et al., 2014). To target a specific gene in mouse embryonic stem (ES) cells, the positive selection gene pGK-neo-polyA is inserted into the targeted locus. The "neo"-cassette can be non-excisable or excisable by flanking 2 repeated loxP (floxed) or flippase recognition target (FRT) sequences (FRT-flanked). The excisable cassette is a better choice, because on Cre- or Flp-mediated DNA recombination, an artificial DNA sequence (e.g., pGK-neo-polyA) in the replaced locus can be eliminated in the ES cells or in the offspring of the gene-targeted mutants. This technique, combining a Cre-loxP and Flp-FRT recombination system, is essential for generating a clean conditional knockout mouse. A reporter gene such as green fluorescence protein (GFP) or β-galactosidase (LacZ) can be used to simplify later tissue-expression mapping in living animals. In the mapping study, strategies to knock in a reporter could face problems when levels of the target genes are too low for detection. Insertion of Cre or Cre-ERT2 recombinase into the target locus might be an alternative to trace target gene expression *in vivo* because many different Cre reporter mice are available. The advantage of using Cre reporter mice is to target a STOP-floxed reporter cassette into a ubiquitously expressing gene locus. With minimal Cre expression, the expression of the targeted, Cre-knockin, gene can be identified. A possible drawback of this strategy is that the enhanced expression of the reporter cannot reflect the up-and-down gene expression during mouse development. Once the gene is turned on, even with minimal expression, during a single developmental stage, the reporter is always "on" throughout the animal's life.

### 2.1. *Asic1*-knockout mice

The exon compositions of *Asic1a* and *Asic1b* are illustrated in Fig. 2A; the 2 transcript isoforms of *Accn2* are generated via different promoters and result in different compositions of the first 2 exons. Wemmie and colleagues replaced exon 2 of *Asic1a* with an inverted neo-cassette, which resulted in a pure *Asic1a*-knockout mouse model (Wemmie et al., 2002). As the first reported mutant mouse strain, it has become the most popular *Asic1a*-knockout model used. Staniland and McMahon (2009) reported another *Asic1*-knockout mouse line created with a LacZ neo-cassette targeted to exons 6–8 of *Asic1a* (or equal to exons 4–6 of *Asic1b*) (Staniland and McMahon, 2009). This knockout design disrupts the transcription of both *Asic1a* and *Asic1b*. Wu and colleagues reported the first conditional *Asic1a*-knockout mouse strain, in which exons 2 and 3 were floxed and the expression level of *Asic1a* mRNA in the neural tissues was equivalent to the wild type (Wu et al., 2013). After crossing with a protamine-Cre transgenic mouse line, the *Asic1a* transcripts in the dorsal root ganglion (DRG) were absent. Therefore, insertion of 2 loxP sites into introns 1 and 3 did not impede endogenous transcription of *Asic1a*, and the floxed exons of *Asic1a* are excisable on Cre recombination (unpublished data from our laboratory). One concern with the genetic manipulation of different *Asic1a* knockout strategies may be the gene compensation in the other splice isoform of *Accn2*, *Asic1b*. Northern blot results of neo-knockin/*Asic1a*-knockout brains were negative (Wemmie et al., 2002), but recent RT-PCR and western blot results of the neo-knockin/*Asic1a*-knockout cerebellum were positive (Vig et al., 2014): endogenous expression of *Asic1b* in the cerebellum was not detected in the wild type but greatly increased in the *Asic1a* knockout.

### 2.2. *Asic2*-knockout mice

The exon compositions of *Asic2a* and *Asic2b* are illustrated in Fig. 2B; the 2 splice isoforms of *Accn1* gene are generated via

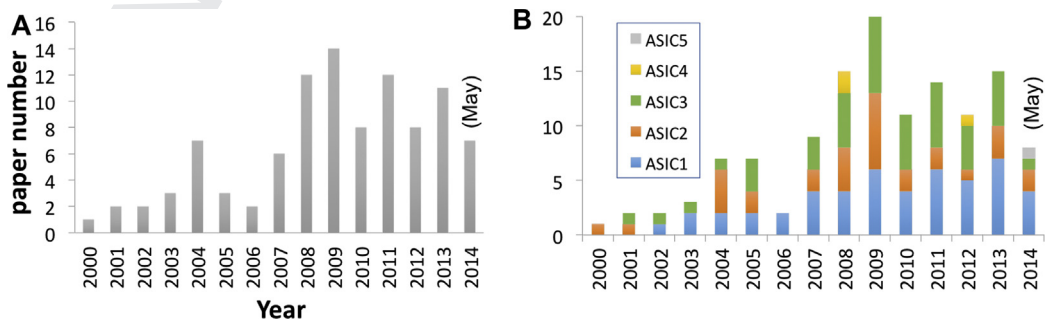


**Fig. 1.** Articles involving ASIC genetically modified mouse models published between 2000 and 2014. (A) The first knockout mouse models for *Asic1a*, *Asic2* and *Asic3* were described in 2002, 2000 and 2001, respectively. In the past few years, studies involving these *Asic* mutant mice have been increasing. (B) Number of published papers by study results in terms of a given ASIC subtype. Most ASIC studies focused on ASIC1, ASIC2 and ASIC3.



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