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Research report

The mouse *Engrailed* genes: A window into autism

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Abstract

The complex behavioral symptoms and neuroanatomical abnormalities observed in autistic individuals strongly suggest a multi-factorial basis for this perplexing disease. Although not the perfect model, we believe the *Engrailed* genes provide an invaluable "window" into the elusive etiology of autism spectrum disorder. The *Engrailed-2* gene has been associated with autism in genetic linkage studies. The *En2* knock-out mouse harbors cerebellar abnormalities that are similar to those found in autistic individuals and, as we report here, has a distinct anterior shift in the position of the amygdala in the cerebral cortex. Our initial analysis of background effects in the *En1* mouse knock-out provides insight as to possible molecular mechanisms and gender differences associated with autism. These findings further the connection between *Engrailed* and autism and provide new avenues to explore in the ongoing study of the biological basis of this multifaceted disease.

Keywords: Autism; *Engrailed*; Amygdala; Cerebellum; Mouse model; Brain development

1. Introduction

Autism spectrum disorder (ASD) is a major mental health disorder afflicting up to 13 out of every 10,000 individuals [29]. Features that typify the broad range of autistic behavior include language impairments (including deficits in verbal and non-verbal communication), restricted patterns of interests and activities, abnormal responses to sensory stimuli, poor eye contact, an insistence on sameness, an unusual capacity for rote memorization, and often repetitive actions [38]. The complexity of the presentation of this malady, however, was perhaps best described by Bachevalier [5] as: "the disruption of a basic characteristic of the human species: the sophistication to generate complex displays of emotion and the ability to respond to the expressive behaviors of other individuals". Determining the biological basis for these behaviors and neurological impairments has remained both elusive and perplexing. Unlike the situation in Parkinson's or Alzheimer's disease, a well-defined collection of major disease genes is not yet available. No clear biomarker or tightly linked change in neurochemistry is known. Finally, the hunts for both neuroimaging signals and neuropathological changes associated with autism have produced highly variable findings with no agreed upon structural alteration emerging. Although the neuroanatomical basis of autism is still somewhat unclear, certain brain regions appear to be regularly altered in individuals with ASD. These include areas in the neocortex, cerebellum, amygdala, hippocampus and brain stem [10,11,19].

One view of ASD that fits with much of the current data is that a timely environmental insult transforms a latent genetic susceptibility into structural abnormalities during the development of the brain. Although the exact nature of the possible environmental contributions remains quite speculative, there is a large body of evidence that indicates that there is a strong genetic component to ASD. For unknown reasons, it affects males four times more frequently than females [30] and a number of reports show significant concordance rates (up to 82%) in monozygotic twins [6,28,61]. Although it is believed that there are a half-dozen or more genes remaining to be discovered, three genes that are emerging as plausible players in the etiology of ASD

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are *reelin* (*RELN*), the *serotonin transporter gene* (*5HTT*), and *Engrailed-2* (*EN2*) [9].

For some time we have been intrigued with the prospect that the *Engrailed* mouse mutants are potentially important tools in the investigation of the biology of autism. It should come as no surprise that virtually any human disease with a complex spectrum of behavioral and biological traits such as autism would be difficult to model in any one animal system. As such, the *Engrailed* genes are not likely to be the sole causal elements in ASD. We prefer to think of the mouse *Engrailed* genes as a "window" into the neurobiological basis of autistic symptoms rather than a direct phenocopy, or even a formal "model" of them. Seen in this light, *Engrailed* seems to capture many aspects of the autistic brain and has the potential to unveil many more. To date, three genetic studies have linked the human *En2* gene to autism [13,32,51]. These studies concur with reports that describe the broad similarities between the neuropathology of the *En2*$^{-/-}$ mouse and a subset of features commonly found in autism. For example, in the *En2*$^{-/-}$ mouse there is a modest yet reproducible disruption in the anterior/posterior pattern of cerebellar foliation and transgene expression, particularly in posterior vermis [37,43]. The folial abnormalities are similar to the distortions seen in autistic individuals as reported by Courchesne et al. [23]. There is also a significant decrease in the number of Purkinje, granule, deep nuclear and inferior olive cells in the *En2* mutant [40]. This again is reminiscent of previously reported neuroanatomical abnormalities observed in autistic individuals [10,11].

Outward phenotypic effects such as these are likely to be the manifestation of a greater core problem. In this regard, we agree with Herbert et al. [35] who proposed that the perturbation of the balance of various brain regions lies at the root of the behavioral symptoms in ASD. One likely location for the fulcrum of this balance is the midbrain/hindbrain boundary of the early neural tube. The *Engrailed* genes are well-described players in the establishment of this area of the nervous system. *En1* is first expressed in a strong transverse band at the junction between the mesencephalon and metencephalon at the one somite developmental stage. A null mutation of *En1* results in the absence of most of the cerebellum and midbrain, and in lethality shortly after birth [66]. *En2* expression is also found at the presumptive midbrain-hindbrain region but initiates later, at the 5 somite stage [25,26]. Unlike *En1*, null alleles of *Engrailed-2* have only a subtle neurological phenotype. There is a developmental change in the banded organization of spinal cord mossy fiber afferents [65] and in the transient pattern of banded expression of *En1*, *En2*, *Pax2*, and *Wnt7b* seen during late gestation [42]. Other markers of cerebellar compartmentation are also disrupted in both the vermis and hemispheres of the *En2*^{hd} null mutant. These include Zebrin II, Ppath and the expression of a *L7lacZ* fusion gene [40].

The *Engrailed* genes are commonly known for their pattern formation activity in the midbrain and hindbrain regions (the only CNS regions where they are expressed), yet both the symptomatology and the structural changes in autism point to significant involvement of telencephalic structures. Some of these include neocortical malformations such as irregular laminar patterns, thickened cortices, abnormally oriented pyramidal cells, and an increase in the number of layer one neurons [7]. In specific cortical areas, Casanova et al. [17] have reported the presence of smaller, more compact, and more numerous minicolumns. Regions in the forebrain that show reduced neuronal size and increased cell packing density include the hippocampus, subiculum, entorhinal cortex, mammillary bodies, anterior cingulated gyrus, septum and amygdala ([38] and see [12,27,47] for recent reviews on autism neuropathology).

Perhaps due to the more posterior location of *Engrailed* expression, none of the telencephalic structures affected in autism have been analyzed in either *Engrailed* mutant. One particularly intriguing location to examine would be the amygdala, as it is an important constituent of the complex neural network of interconnected structures that give rise to certain aspects of social behavior. The amygdala is located at the medial edge of

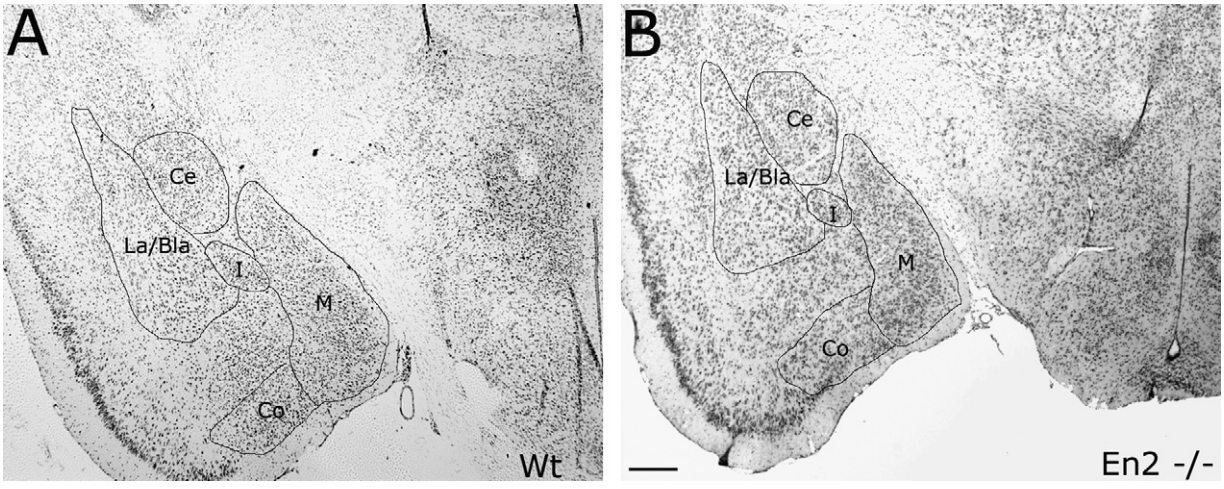

Fig. 1. Amygdalar subdivisions in *En2*$^{-/-}$ and control sections. Cresyl violet-stained, 10 μm representative sections in the coronal aspect from control (A) and *En2* mutant (B) brains from 3-month-old male mice indicate the marked similarity in amygdalar subdivisions: central, medial, and lateral/basolateral nuclei. The intermediate nucleus and the cortical nucleus are also quite conspicuous and serve as a reference for slide alignment. Ce, central nucleus; Co, cortical nucleus; M, medial nucleus; La/Bla, lateral and basolateral nucleus; I, intermediate nucleus. Scale bar equals 0.2 mm.