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Research Report

Modified sound-evoked brainstem potentials in *Foxp2* mutant mice

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ABSTRACT

Heterozygous mutations of the human *FOXP2* gene cause a developmental disorder involving impaired learning and production of fluent spoken language. Previous investigations of its aetiology have focused on disturbed function of neural circuits involved in motor control. However, *Foxp2* expression has been found in the cochlea and auditory brain centers and deficits in auditory processing could contribute to difficulties in speech learning and production. Here, we recorded auditory brainstem responses (ABR) to assess two heterozygous mouse models carrying distinct *Foxp2* point mutations matching those found in humans with *FOXP2*-related speech/language impairment. Mice which carry a *Foxp2-S321X* nonsense mutation, yielding reduced dosage of Foxp2 protein, did not show systematic ABR differences from wildtype littermates. Given that speech/language disorders are observed in heterozygous humans with similar nonsense mutations (*FOXP2-R328X*), our findings suggest that auditory processing deficits up to the midbrain level are not causative for *FOXP2*-related language impairments. Interestingly, however, mice harboring a *Foxp2-R552H* missense mutation displayed systematic alterations in ABR waves with longer latencies (significant for waves I, III, IV) and smaller amplitudes (significant for waves I, IV) suggesting that either the synchrony of synaptic transmission in the cochlea and in auditory brainstem centers is affected, or fewer auditory nerve fibers and fewer neurons in auditory brainstem centers are activated compared to wildtypes. Therefore, the *R552H* mutation uncovers possible roles for *Foxp2* in the development and/or function of the auditory system. Since ABR audiometry is easily accessible in humans, our data call for systematic testing of auditory functions in humans with *FOXP2* mutations.

1. Introduction

Heterozygous mutations of the *FOXP2* gene cause the best described example of an inherited speech and language disorder in humans (Lai et al., 2001; MacDermot et al., 2005). It is characterized by impaired learning/production of complex oral movements underlying speech, accompanied by linguistic deficits which affect both spoken and written modalities (Watkins et al., 2002; see Fisher, 2006 for review). *FOXP2* encodes a forkhead-box transcription factor with a

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Abbreviations: ABR, auditory brainstem response; ENU, N-ethyl-N-nitrosourea; Foxp2, forkhead-box p2; SPL, sound pressure level; WT, wildtype

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characteristic DNA-binding domain, and acts to regulate expression of many downstream target genes (Vernes et al., 2007). The gene is found in highly similar form in many vertebrate species, including rodents, where it is expressed in corresponding neuronal subpopulations of the brain (Fisher and Scharff, 2009). Recently, an ENU (*N*-ethyl-*N*-nitrosourea) mutagenesis strategy enabled the generation of mouse models carrying distinct *Foxp2* point mutations (single nucleotide changes) that match those observed in humans with speech/language deficits (Groszer et al., 2008).

Foxp2-R552H mice recapitulate the human *FOXP2-R553H* missense mutation found in a particularly well-studied three-generation family, known as the KE family; this yields an amino-acid substitution in the encoded protein, replacing an arginine residue with a histidine at one crucial site within the DNA-binding domain (Lai et al., 2001). In cell-based studies, the mutated protein shows abnormal intracellular localization, impaired DNA-binding and disturbed regulation of transcriptional activity of targets (Vernes et al., 2006). *Foxp2-S321X* mice carry a different type of point mutation, known as a nonsense mutation, which creates a premature stop codon midway through the gene, and is thus expected to encode a truncated protein lacking the DNA-binding domain and other key functional regions. In fact, *in vivo* analyses indicate that this allele does not produce Foxp2 protein at all, most likely due to a combination of nonsense-mediated RNA decay (degradation of mRNA transcripts carrying the nonsense mutation) and instability of any truncated protein that does get produced (Groszer et al., 2008; Vernes et al., 2006). The *Foxp2-S321X* mutation is very similar to a human *FOXP2-R328X* nonsense mutation found in all three affected members of another family segregating speech and language deficits (MacDermot et al. 2005).

Mice that are homozygous for the above mutations display severe reductions in cerebellar growth and postnatal weight gain, as well as profound general motor impairment, and die after 3–4 weeks. Heterozygous mice are fully viable and gain weight normally, but show deficits in motor-skill learning and synaptic plasticity, including a lack of long-term depression in the dorsolateral striatum (Groszer et al., 2008). Both heterozygous and homozygous pups produce innate ultrasonic vocalizations despite *Foxp2* disruption (Groszer et al., 2008).

Speech learning and production make heavy demands on rapid and fine motor control, but also depend on sensory processing within the auditory system (Fitch et al., 1997; Smith and Spirou, 2002). Thus, deficits in such sensory pathways could potentially contribute to impaired speech and language development (Hill et al., 2005). People carrying *FOXP2* mutations have been reported to have overtly normal hearing (Hurst et al., 1990). However, to our knowledge, no formal quantitative assessments of auditory abilities have yet been carried out, either in humans with *FOXP2*-related speech/language disorder, or in *Foxp2* mutant mice. Therefore, we used auditory brainstem response (ABR) audiometry which employs scalp electrodes to record sound-evoked bioelectrical potentials and to assess the peripheral auditory function of heterozygous *Foxp2* mutant mice in comparison to wildtype (WT) littermates. The ABR method has widely been applied to identify hearing deficits related to the auditory pathway from the cochlea up to the auditory midbrain in mice of various genetic backgrounds (e.g. Shvarev, 1994; Willott et al., 1995; Trune et al., 1996; Reimer et al., 1996; Zheng et al., 1999; Burkard et al., 2001). Five peaks in the ABR waves are expected. In the mouse, peak I is suggested to refer to cochlear processing, peak II to processing in the cochlear nucleus complex, peak III in the complex of the superior olive, peak IV in the lateral lemniscus and peak V in the colliculus inferior.

In this context, physiological investigations of mouse models with *Foxp2* point mutations matching those that cause human speech/language impairment provide a valuable opportunity to identify novel aspects of aetiology in this disorder, and shed new light on functions of the gene. Furthermore since ABR measurements can easily be employed in humans, this method is useful for model validation and cross species comparisons.

2. Results

The analyses of hearing sensitivity (ABR thresholds), and amplitudes and latencies of the five peaks of the ABR waves identified differences in sound processing in the auditory periphery and brainstem of the *Foxp2* mutant mice. In Fig. 1 examples of the mean ABR of *R552H* and *S321X* heterozygous mutants and corresponding WT littermates are shown in response to 16 kHz tone bursts at a sound pressure level set to 20 dB above threshold. Five response peaks (I–V) with different latencies could be identified for all mice tested. No obvious

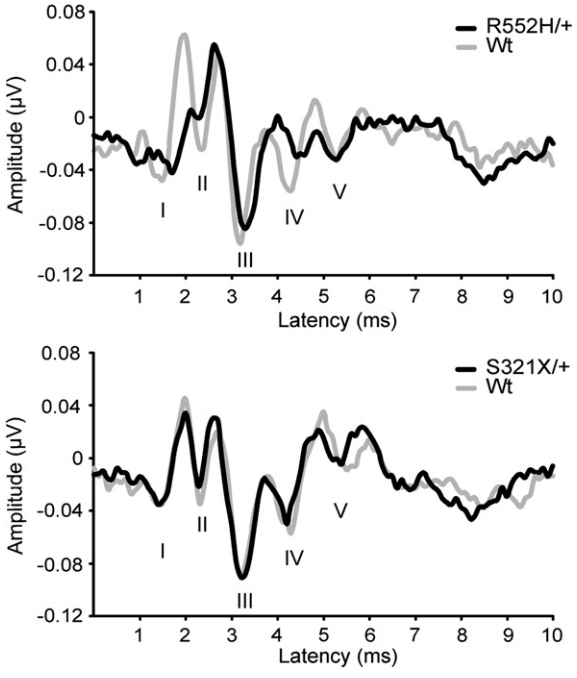

Fig. 1 – Examples of mean ABR recordings of *R552H* and *S321X* heterozygotes in comparison with their WT littermates. All recordings shown were obtained with a standard stimulus of 16 kHz tone bursts at 20 dB above response threshold. Recordings start with sound arrival at the ear (0 ms). Roman numbers indicate peaks I to V of the ABR waves.