Neuroscience 175 (2011) 75–84

HUMANIZED Foxp2 SPECIFICALLY AFFECTS CORTICO-BASAL GANGLIA CIRCUITS

S. REIMERS-KIPPING, W. HEVERS, S. PÄÄBO AND W. ENARD*

Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, D-04103 Leipzig, Germany

Abstract—It has been proposed that two amino acid substitutions in the transcription factor FOXP2 have been positively selected during human evolution and influence aspects of speech and language. Recently it was shown that when these substitutions are introduced into the endogenous *Foxp2* gene of mice, they increase dendrite length and long-term depression (LTD) in medium spiny neurons of the striatum. Here we investigated if these effects are found in other brain regions. We found that neurons in the cerebral cortex, the thalamus and the striatum have increased dendrite lengths in the humanized mice whereas neurons in the amygdala and the cerebellum do not. In agreement with previous work we found increased LTD in medium spiny neurons, but did not detect alterations of synaptic plasticity in Purkinje cells. We conclude that although Foxp2 is expressed in many brain regions and has multiple roles during mammalian development, the evolutionary changes that occurred in the protein in human ancestors specifically affect brain regions that are connected via cortico-basal ganglia circuits. **© 2011 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: FOXP2, dendrite length, synaptic plasticity, evolution, pleiotropy.

The genetic basis of human evolution is of cultural as well as medical interest (Enard and Paabo, 2004; Varki et al., 2008). There are approximately 20 million nucleotide sequence differences between currently living humans and the last common ancestor that humans shared with chimpanzees some 6 million years ago (Mikkelsen et al., 2005). Among these "human-specific" changes must be those that built the genetic basis for human abilities that evolved during this time, such as the ability to acquire language and to learn the complex motor movements necessary for speech. Almost nothing is known about this link, but two human-specific amino acid changes in the transcription factor FOXP2 are intriguing candidates for two reasons.

First, FOXP2 is currently the only gene for which a specific link to the development of speech and language is well established (Vargha-Khadem et al., 2005; Fisher and Scharff, 2009). Humans carrying only one functional copy of the gene (Lai et al., 2001; MacDermot et al., 2005) have difficulties in performing complex movements of the mouth and face, show impairments in expressive and receptive language tasks, but show hardly any impairments in other cognitive skills (Vargha-Khadem et al., 1998; Watkins et al., 2002a). Brain imaging studies suggest that the deficits are due to functional impairments in the cortex, the striatum and/or the cerebellum (Vargha-Khadem et al., 1998; Watkins et al., 2002b; Belton et al., 2003; Liegeois et al., 2003). It has been argued that the patients resemble a developmental variant of aphasias that are caused, for example, by lesions of Broca's area (Watkins et al., 2002a).

Second, two amino acid substitutions have occurred during 6 million years of recent human evolution, which is more than expected for a protein that differs only by one amino acid substitution between chimpanzee and mouse. This suggests that FOXP2 has been positively selected specifically during human evolution (Enard et al., 2002; Zhang et al., 2002). This is an intriguing correlation with the emergence of language and speech in humans, but to understand the functional consequences of these evolutionary changes it is necessary to compare the human version of FOXP2 with non-human versions in a relevant model system. We recently took a first step in this direction by creating a mouse in which the two amino acid changes in Foxp2 have been knocked-in (Enard et al., 2009). These mice, "humanized" for Foxp2, are generally healthy and show no phenotypic effects in many organ systems in which Foxp2 is expressed. However, they do show slight changes in pup vocalizations, decreased exploratory behavior, and decreased dopamine concentrations. Furthermore, medium spiny neurons in the striatum have increased dendrite lengths *in vitro* and *in vivo* and show stronger long-term depression (LTD) after high-frequency stimulation. Interestingly, mice heterozygous for non-functional alleles of *Foxp2* show partly opposite effects (Groszer et al., 2008; Enard et al., 2009). An important unanswered question is to what extend the effects of humanized Foxp2 are specific to particular neurons.

Foxp2 is expressed in many cells throughout the body (Lai et al., 2001; Shu et al., 2005, 2007). In the brain it is expressed in a subset of postmitotic neurons, such as in medium spiny neurons, Purkinje cells, neurons of cortical layer VI, and thalamic neurons, but it is, for example, not expressed in hippocampal neurons (Ferland et al., 2003; Lai et al., 2003; Campbell et al., 2009). Here we investigate the neuroanatomical and electrophysiological effects in different brain regions of the humanized *Foxp2* mice in order to better understand the consequences of the two amino acid changes.

*Corresponding author. Tel: +49-3413550511; fax: +49-3413550555.
E-mail address: enard@eva.mpg.de (W. Enard).
Abbreviations: ANOVA, analysis of variance; LTD, long-term depression; PBS, phosphate buffered saline; TBST, tris buffered saline Tween 20.

0306-4522/11 $ - see front matter © 2011 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2010.11.042

EXPERIMENTAL PROCEDURES

Mice

The investigated humanized *Foxp2* (*Foxp2*^{hum}) allele is the *Foxp2*$^{hum\Delta neo}$ allele derived from the embryonic stem (ES) cell clone 5H11 described in (Enard et al., 2009). The *Foxp2* knockout (*Foxp2*^{ko}) allele is derived from the same ES cell clone and results in a premature stop codon in exon 8 and absence of Foxp2 protein in Western blot analyses (Enard et al., 2009). Since the ES cell line is derived from C57BL/6 mice and all breedings were done with C57BL/6J mice, the genetic background can be considered as pure C57BL/6J. *Foxp2*$^{hum/hum}$ and *Foxp2* wildtype (*Foxp2*$^{wt/wt}$) mice were derived from heterozygous crossings and balanced across litters. Mice were housed at a 10 h/14 h dark/light cycle under standard conditions. All animal work was performed in accordance with governmental and institutional guidelines. All efforts were made to minimize the number of animals used and their suffering.

Immunohistochemistry

Foxp2$^{ko/ko}$ and *Foxp2*$^{wt/wt}$ embryonic heads were obtained from heterozygous crossings 17.5 days after mating (E17.5) and fixed with 4% paraformaldehyde in phosphate buffered saline (PBS) pH 7.4 at 4 °C for 24 h. Adult *Foxp2*$^{hum/hum}$ and *Foxp2*$^{wt/wt}$ animals (12 weeks, for all brain regions except cerebellum) and postnatal day 17 (P17; only for cerebellum) animals were perfused with 4% paraformaldehyde in PBS, their brains dissected and postfixed with the same solution for 24 h at 4 °C. Brains were dehydrated in 15% and subsequently in 30% sucrose in PBS for 24 h at 4 °C, embedded in Tissue-Tek OCT compound (Sakura Japan) and cut into 40 μm coronal slices using a cryomicrotome (Microm HM550, Thermo Scientific, Walldorf, Germany). Series of every 10th section were incubated floatingly (adult and P17) or mounted on glass slides (embryonic) for 10 min with an antigen unmasking buffer (10 mM sodium citrate buffer pH 6.0) at 98 °C, cooled for 30 min at room temperature and washed three times with bi-distilled H_2O, three times with Tris buffered saline with 0.1% Tween 20 pH 7.4 (TBST) and afterward blocked for 2 h with 5% normal donkey serum (NDS) and 1% bovine serum albumin (BSA) in TBST. Brain sections were incubated overnight with the primary antibody in the blocking buffer (rabbit anti-Foxp2 antibody HPA000382, Atlas Antibodies, Stockholm, Sweden; 0.12 μg/ml), chicken anti-MAP2 ab5392 (Abcam, Cambridge, UK; 1.9 μg/ml), mouse anti-NeuN biotinylated ab77315 (Abcam; 2 μg/ml), goat anti-DARPP32 sc-8483 (Santa Cruz Biotechnologies Inc., Santa Cruz, CA, USA; 4 μg/ml), mouse anti-Calbindin D-28K, C9848, (Sigma-Aldrich, St. Louis, MO, USA; 3.2 μg/ml), washed three times with TBST and incubated for 8 h with secondary antibodies in blocking buffer (DyLightTM 549-conjugated AffinityPure *F*(ab′)2 Fragment Donkey anti-Chicken IgY++ (H+L); 2 μg/ml, Biotin-SP-conjugated AffiniPure Donkey anti-goat++ IgG (H+L); 2 μg/ml), Biotin-SP-conjugated AffiniPure Donkey anti-mouse; 2 μg/ml, Biotin-SP-conjugated AffiniPure Donkey anti-rabbit (2 μg/ml; all purchased from Jackson ImmunoResearch, West Grove, PA, USA). Sections were washed with TBST three times, incubated over night with streptavidin DyLight conjugates in blocking buffer (1 μg/ml; Jackson ImmunoResearch), washed with TBST twice and once with TBS and subsequently mounted on glass slides using Prolong Antifade (Invitrogen, Paisley, UK). Laser scanning microscopy (LSM 510; Zeiss, Jena, Germany) was performed with appropriate emission and excitation filters for maximal signal separation. For all co-localizations of Foxp2 with neuronal markers, images were acquired at constant exposure times and pinhole settings for all animals and brain regions to ensure comparability. During data collection, the investigator was blind to the genotype of the animals.

Quantification of Foxp2-expressing cells

Foxp2 co-localization with the neuronal marker proteins NeuN (in neocortex, piriform cortex, thalamus, and striatum) and calbindin (in cerebellum) were analyzed quantitatively to determine the fraction of Foxp2-expressing neurons in these regions. Serial sections of three adult *Foxp2*$^{hum/hum}$ and *Foxp2*$^{wt/wt}$ mice were triple labeled with anti-Foxp2 (Atlas Antibodies), anti-MAP2 and anti-NeuN or anti-calbindin antibodies. Images were acquired as described above. 100 NeuN-positive neuronal nuclei and 40 calbindin-positive Purkinje cell bodies were identified in at least four individual images per animal using the particle analyzer tool of the ImageJ software. To obtain absolute numbers of Foxp2-positive neurons, Foxp2 immunoreactivity was scored as positive for the NeuN or calbindin-positive cell bodies, if the cell body exhibited intact morphology and nuclear FOXP2 staining. Numbers of Foxp2-positive neurons were averaged per animal and the mean calculated across all animals of the same genotype to determine percent co-localization. During data collection, the investigator was blind to the genotype of the animals.

Analysis of dendrite length

For the analysis of dendrite lengths in neurons of the thalamus, the amygdala, the neocortex and the piriform cortex we examined brains of six adult *Foxp2*$^{hum/hum}$ and six adult *Foxp2*$^{wt/wt}$ littermates, previously stained using the FD Rapid GolgiStain™ kit (FD NeuroTechnologies, Ellicott City, MD, USA) as described (Enard et al., 2009). Total dendritic trees were tracked in 200 μm thick coronal slices using an oil-immersion lense (10×63.5) and Neurolucida software (MBF Bioscience, Williston, ND, USA). Being blind to genotype, we measured three cells per region and animal in five of the six animals per genotype. Only neurons whose dendritic trees were located completely within the section were included in the analysis. For the analysis of dendrites in Purkinje cells, P17 mouse brains were used to ensure the presence of all dendritic arborizations and processed in the same way. Dendrites of three cells per animal were tracked in 200 μm sagittal slices. Measurements of medium spiny neurons, located throughout the striatum, were taken from Enard et al., 2009. All 289 measurements are available upon request. We analyzed the data using SPSS v.16.0 (SPSS Inc., Chicago, IL, USA). For analysis of variances (ANOVAs) we used Levene's test for homoscedasticity and visual inspection of residuals plotted against predicted values to check for violations of assumptions. Since we only tested for an increased length of dendrites in *Foxp2*$^{hum/hum}$ mice we report one-sided *P*-values for all genotype-dependent effects. For analyzing all cell types we used the following model: Log_2(total dendritic length)=Genotype+*Foxp2expression*+*CellType(Foxp2expression)*+*Genotype*Foxp2expression*+*Genotype*CellType(Foxp2expression)*+*error*, whereas the factor *Genotype* has two levels (*Foxp2*$^{hum/hum}$ and *Foxp2*$^{wt/wt}$), the factor *Foxp2expression* has two levels (yes when over 20% of the neurons are positive for Foxp2) and the factor *CellType* has 10 levels (the 10 examined neuronal cell types). When analyzing only cell types expressing Foxp2 the same model without the factor *Foxp2expression* was used. When considering the factor *Circuit* (with level yes for the two Foxp2 expressing cell types in layer VI, striatal neurons and thalamic neurons) for these cells, the following model was applied: Log_2(total dendritic length)=Genotype+Circuit+*CellType(Circuit)*+*Genotype*Circuit*+*Genotype*CellType (Circuit)*+*error*. Note that these models assume independence of each measured neuron, despite the fact that the same animals were used for different cell types. The violation of this assumption does not seem to be very problematic because within the same animals we observe no significant correlations of dendritic length across cell types and only very limited correlation within the same cell types (data not shown). Nevertheless, using a fully balanced dataset of four *Foxp2*$^{hum/hum}$ animals and five *Foxp2*$^{wt/wt}$ animals for which neurons had been measured in all cell types, we ran an ANOVA with animal as random factor that takes into account this dependency: Log_2

Download English Version:

https://daneshyari.com/en/article/6276567

Download Persian Version:

https://daneshyari.com/article/6276567

Daneshyari.com