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Research Report

Ongoing expression of Nkx2.1 in the postnatal mouse forebrain: Potential for understanding *NKX2.1* haploinsufficiency in humans?

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ABSTRACT

Coordinated movements require the caudate–putamen and the globus pallidus, two nuclei belonging to the basal ganglia, to be intact and functioning properly. Many neurons populating these regions derive from the medial ganglionic eminence, a transient structure that expresses the transcription factor Nkx2.1 during prenatal development. Accordingly, the basal ganglia of *Nkx2.1*$^{-/-}$ mice are heavily affected and a substantial loss of several types of GABAergic interneurons has been observed. Interestingly, heterozygous mutation of the *NKX2.1* gene in humans has been described as causing an unusual disorder from the second year of life onwards, which is mainly characterized by disturbances of motor abilities and delayed speech development. In the present study, we therefore investigated whether Nkx2.1 is still expressed in the young adult and aged mouse forebrain. After birth, the most intense immunolabeling for Nkx2.1 was detected in several components of the hypothalamic region, in the subventricular zone of the ventral tips lining the lateral ventricles, and in neighboring structures including the striatum, the globus pallidus and the various nuclei of the septal complex. Surprisingly, this staining pattern was substantially maintained into adulthood. Double immunocytochemistry for Nkx2.1 and various neuronal markers revealed that mainly parvalbumin-containing GABAergic neurons, but also cholinergic neurons, of the ventral forebrain express this protein. Moreover, in situ hybridization confirmed that these neurons maintain synthesis of Nkx2.1 throughout life. The robust expression of Nkx2.1 by these neurons points to a broad functional spectrum within the adult forebrain.

1. Introduction

Haploinsufficiency is a rare genetic phenomenon related to semidominant genes. Since these genes only fulfill their normal function in the presence of both wild-type alleles, heterozygous loss of function mutations lead to phenotypic abnormalities (for review, see Nutt and Busslinger, 1999). For instance, haploinsufficiency of the transcription factor *NKX2.1*

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in humans specifically results in dysfunctions of the thyroid gland, the lungs, and the brain (Iwatani et al., 2000; Breedveld et al., 2002a,b; Krude et al., 2002; Doyle et al., 2004; Asmus et al., 2005; do Carmo Costa et al., 2005; Moya et al., 2006; Devos et al., 2006; Provenzano et al., 2008; for review, see: Kleiner-Fisman and Lang, 2007). This "brain–thyroid–lung syndrome" emerges in early childhood and displays a variable combination of symptoms including hypothyroidism, respiratory distress, apneic episodes, and choreoathetosis (Krude et al., 2002; Willemsen et al., 2005). The latter is characterized by delayed development of motor abilities, persistent ataxia, dysarthria, muscular hypotonia, hyperextendable knee joints, muscular atrophy of the lower limbs, and rapid choreatic and athetotic movements of the limbs, face and trunk. The severity of the neurological symptoms varies between the patients and mostly depends on the type of mutation affecting the *NKX2.1* gene. However, in most cases the symptoms become less severe or even disappear once adulthood is reached. Interestingly, no psychiatric or cognitive abnormalities have been found so far in most of the patients, and only some individuals fail to finish school and/or show mild mental abnormalities (Breedveld et al., 2002a; Willemsen et al., 2005; Moya et al., 2006). Thus, most of the neurological deficits reflect a disturbed development and/or maturation of the motor functions and are most likely associated with malformations of the basal ganglia (Krude et al., 2002; do Carmo Costa et al., 2005).

Nkx2.1 was originally shown to regulate the expression of two thyroid genes, and hence, this protein is also called thyroid transcription factor 1 (TTF1) or thyroid enhancer binding protein (T/EBP; Civitareale et al., 1989; Mizuno et al., 1991). It belongs to the homeobox gene family, and is translated into a protein closely related to members of the Drosophila NK-2 homeodomain protein family (Guazzi et al., 1990). During development of vertebrates, Nkx2.1 is expressed in the Anlagen of the thyroid gland and lungs but also in certain forebrain regions (Lazzaro et al., 1991; Price et al., 1992). In the mouse brain, *Nkx2.1* mRNA was first detected at embryonic stage E8.75 (1-somite-stage according to Shimamura et al., 1995). At E10 strong mRNA expression was observed in the hypothalamic primordium, the medial ganglionic eminence (MGE), and ventral parts of the immature telencephalon which give rise to the septal complex, the anterior entopeduncular region and the preoptic area (Sussel et al., 1999; Flames et al., 2007; Garcia-Lopez et al., 2008). Cells produced in each domain (or their smaller subdivisions) migrate either radially to the mantle zone or tangentially to adjacent and distant regions (Marin et al., 2000; Wichterle et al., 2001). The development of the large subcortical compartments formed mainly by projection neurons is thus a result of radial migration. Other precursors deriving from the same proliferative zones tangentially migrate and integrate in these compartments. At their final positions, these cells differentiate into the various types of interneurons which, in general, represent a minor subpopulation. For instance, the MGE gives rise to most of the GABAergic projection neurons in the globus pallidus but also the majority of interneurons in the neighboring caudate–putamen.

The importance of Nkx2.1 for the correct development of these neuronal populations has been demonstrated by the severe phenotype of the *Nkx2.1* null mouse. Homozygous *Nkx2.1*$^{-/-}$ mutants develop only a rudimentary thyroid gland and die directly after birth due to agenesis of the lungs (Kimura et al., 1996). However, severe morphological alterations were already found in the prenatal brain: the globus pallidus is missing while the striatum appears to be enlarged. In addition, certain nuclei of the hypothalamic area are heavily affected or completely missing (Kimura et al., 1996). At cellular level, immunohistochemical analysis of prenatal mutants at E18.5 revealed a 40% reduction in the number of glutamate decarboxylase 67 (Gad67)-positive interneurons in the cerebral cortex. In line with this, neuronal cell numbers for hippocampal GABAergic interneurons coexpressing neuropeptide-Y (NPY), calbindin (CB), or somatostatin (SOM) were also found to be significantly lower at E18.5 (Pleasure et al., 2000). Moreover, in the mutant striatum a subtotal loss of interneurons was observed. Choline acetyltransferase (ChAT)-, parvalbumin (PV)-, SOM-, NPY-, nitric oxide synthase (NOS)-, and calretinin (CR)-positive neurons are either severely reduced or totally absent (Marin et al., 2000). In addition, cholinergic basal forebrain neurons expressing the high affinity nerve growth factor receptor trkA were not detectable (Sussel et al., 1999).

Several studies have shown that the expression of Nkx2.1 extends into the postnatal life of rodents. As demonstrated for mice, Nkx2.1 is not only necessary for the maintenance of the normal architecture and function of the differentiated thyroid gland (Kusakabe et al., 2006), but also for the hypothalamic control of puberty and reproductive function (Mastronardi et al., 2006). Moreover, several reports have indicated that Nkx2.1 synthesis is also maintained in other basal forebrain regions (Marin et al., 2000; Lee et al., 2001; Nakamura et al., 2001; Kim et al., 2006; Xu et al., 2008). However, these studies have only been performed for up to 3 weeks after birth, and they can therefore not explain the sensitivity of motor functions to *NKX2.1* haploinsufficiency in humans. In fact, both the delay in speech development and the persisting defects in movement coordination suggest that long-lasting expression of NKX2.1 in the basal ganglia is needed for the sufficient maturation of these abilities. Since a detailed characterization of subcortical Nkx2.1-expressing neurons has not been performed yet for the adult and aged mouse, we used immunocytochemistry and in situ hybridization to identify Nkx2.1-positive cells in the basal forebrain. Here we show that many neurons of both the basal ganglia and septal complex, but also several neighboring regions located more ventrally, maintain synthesis of Nkx2.1 throughout life. In addition, Nkx2.1-expression is not only maintained by subcortical GABAergic interneurons but also by GABAergic and cholinergic projection neurons of several basal forebrain nuclei. These so-called "cholinergic centers" play, for instance, an important role for learning and memory processes (Dutar et al., 1995; Heimer et al., 1997; Berger-Sweeney, 2003; Mufson et al., 2003).

2. Results

Neurochemical characterization of neurons in rodents reveals remarkable differences not only between rats and mice but also between strains of the same species (e.g., Tunnicliff et al.,