

## LETTER

# Dysfunctional NF- $\kappa$ B and brain myelin formation

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In a recent issue of the *European Journal of Human Genetics*, Philippe *et al*<sup>1</sup> reported on a duplication of the Xq28 locus and its supposed contribution to distorted brain myelination in patients carrying this mutation. They provided evidence that additional copies of the *IKBKG* gene (encoding *NEMO*, the regulatory subunit of the IKK complex) functionally impair NF- $\kappa$ B signaling, which in turn leads to the developmental brain abnormalities and mild mental retardation. MRI-based application of axial T<sub>2</sub>-weighted FSE and coronal FLAIR sequences to differentiate between CNS white and gray matter confirmed defective myelination in three out of five patients. The authors also presented convincing data that, as exemplified by isolated fibroblasts, NF- $\kappa$ B-dependent gene expression is indeed impaired in these patients carrying the *IKBKG* mutation.<sup>1</sup> Accounting for the structural phenotype observed, they concluded that proper myelination requires NF- $\kappa$ B activation in CNS-intrinsic cell populations.

This case study is highly valuable in providing evidence for a contribution of NF- $\kappa$ B to normal neurodevelopment. However, in line of our findings in transgenic mice, the proposal of a direct relationship between Xq28 duplication/*NEMO* hyperactivation in neuro-ectodermal cells and myelination deficits/microcephaly appears questionable. To realize neuro-ectodermal deletion of the transactivating NF- $\kappa$ B subunit RelA (*RelA*<sup>CNSKO</sup>),<sup>2</sup> we crossed mice carrying floxed *relA* alleles with transgenic mice expressing Cre recombinase under the control of the nestin promoter. Displaying almost complete removal of RelA protein from the CNS as verified by immunoblotting (see Supplementary Figure S1), this construct was used to analyze the requirement of RelA for proper myelin formation in the CNS using histological procedures, MR imaging, and behavioral tests. Histological and electron microscopic analyses of the optic nerve showed unimpaired oligodendrocyte densities (wild type, WT: 877.2 ± 27.7 cells/mm<sup>2</sup>, *RelA*<sup>CNSKO</sup>: 851.9 ± 48.4 cells/mm<sup>2</sup>;  $P = 0.43$ ) and normal myelin sheath formation in *RelA*<sup>CNSKO</sup> mice as assessed by g-ratios (WT: 0.71 ± 0.02, *RelA*<sup>CNSKO</sup>: 0.71 ± 0.02;  $P = 0.99$ ). No abnormalities in brain structure were observed in T<sub>1</sub>- and T<sub>2</sub>-weighted sequences obtained in a 3 T magnetic field, and magnetization transfer (MT) sequences excluded reduced myelin contents in the cerebellar white matter of *RelA*<sup>CNSKO</sup> mice (MT ratio WT: 35.8 ± 2.3%, *RelA*<sup>CNSKO</sup>: 34.1 ± 2.9%;  $P = 0.7$ ). Furthermore, tracer-based MR imaging (MEMRI) revealed normal active Mn<sup>2+</sup> uptake into retinal ganglion cells and transport along the retino-tectal projection (signal-to-noise ratio in superior colliculi WT: 69.24 ± 2.02, *RelA*<sup>CNSKO</sup>: 70.52 ± 5.77;  $P = 0.86$ ), indicating unimpaired nerve fiber vitality and function as compared to controls. Likewise, functional parameters of visual acuity and contrast sensitivity were indistinguishable between WT and *RelA*<sup>CNSKO</sup> mice (published elsewhere). These

findings are in line with previous reports showing that mice with inactivated upstream regulators of NF- $\kappa$ B (I $\kappa$ B $\zeta$ , IKK) in the neuro-glial compartment are indiscernible regarding overall neuro-anatomical and behavioral features<sup>3,4</sup> and, in particular, display normal myelination.<sup>5</sup> Among the NF- $\kappa$ B family members (RelA, RelB, c-Rel, p105/50, p100/52), only deletion of the subunit p50, which lacks a transcriptional activator domain, results in a destructive neuronal phenotype as characterized by precocious aging, neuronal apoptosis and spontaneous demyelination in young adult mice.<sup>6</sup> However, even in the case of p50 deletion, disturbances in congenital brain and myelin development have not been described to date. As p50 deficiency, as previously shown by others<sup>7,8</sup> and supported by our own data on NF- $\kappa$ B reporter mice (published elsewhere), results in enhanced rather than diminished NF- $\kappa$ B activity, such a phenotype does not offer a straightforward explanation for the neurodevelopmental deficits described under Xq28-dependent NF- $\kappa$ B inactivation. Nevertheless, RelA deficiency might lead to a compensatory replacement by other subunits, particularly by c-Rel. Indeed, switches in NF- $\kappa$ B dimer composition have been reported for cerebral ischemia.<sup>9</sup> We are currently generating neuro-glia-specific *RelA*;*c-Rel* double-knockout mice to establish whether white matter abnormalities ensue.

Undoubtedly, the highly informative character of the case study by Philippe *et al*<sup>1</sup> highlights the clinical impact of NF- $\kappa$ B for proper neurodevelopment. Their data are supported by previous case studies on deregulated NF- $\kappa$ B signaling in patients carrying mutations in the *TRAPPC9* gene, which encodes the NF- $\kappa$ B-inducing kinase (NIK)- and I $\kappa$ B kinase complex  $\beta$  (IKK- $\beta$ )-binding protein.<sup>10</sup> Although both factors are required for proper activation of NF- $\kappa$ B, *NEMO* and NIK-/IKK- $\beta$ -binding protein might act via NF- $\kappa$ B-independent pathways in the context of myelination. Alternatively, a complex relationship between cerebral myelin formation and NF- $\kappa$ B-dependent gene expression in non-neuro-ectodermal cells might exist. One example of such an interaction is represented by the pathology of incontinentia pigmenti (IP), an X-linked dominant disorder with recurrent mutation in the *NEMO* gene, where about one-third of affected patients develop ocular and neurological deficits including mental retardation, microcephaly, and cerebellar ataxia.<sup>11</sup> The fundamental neuropathology in IP, however, is not a neuro-ectodermal, but a mesenchymal dysfunction of cerebral blood vessels, leading to infantile microvascular ischemia and subsequent neuronal damage.<sup>12</sup>

Taken together, the currently available data from transgenic mice show discrepancies compared to the human phenotype described by Philippe and colleagues.<sup>1</sup> Further research is necessary to clarify whether inhibition of upstream activators of NF- $\kappa$ B or of specific dimer compositions in (non-)neuro-ectodermal cells is of relevance in developmental white matter abnormalities.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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