From mice to man: chloride transport in leukoencephalopathy

In *The Lancet Neurology*, Christel Depienne and colleagues\(^1\) show that mutations in *CLCN2*—the gene encoding the chloride channel CIC-2—underlie a specific form of human leukoencephalopathy. This important work confirms in human beings what was previously shown in CIC-2 knock-out (*Clcn2\(^{-/-}\)*) mice whose leukodystrophy\(^1\) was suggested to result from impaired glial ion homeostasis. It also raises the question as to whether the pathogenesis of human leukoencephalopathies caused by mutations in the cell adhesion molecule GlialCAM\(^1\) or the membrane protein MLC1\(^1\) involve changes in CIC-2.

CIC-2 is expressed almost ubiquitously,\(^5\) including in neurons and glia. *Clcn2\(^{-/-}\)* mice have retinal and testicular degeneration,\(^6\) which were thought to result from impaired ion homeostasis in the extracellular clefts between supporting epithelia and photoreceptors and germ cells, respectively. Because these mice did not have relevant neurological phenotypes, the leukodystrophy was discovered only later.\(^7\) *Clcn2\(^{-/-}\)* mice developed white matter vacuolation in several brain regions with vacuoles forming within myelin sheaths of axons.\(^7\) Knock-out-controlled immunohistochemistry detected CIC-2 in Bergmann glia, astrocytic endfeet adjacent to blood vessels, and on cell bodies of oligodendrocytes where it partially colocalised with the gap junction protein connexin 47.\(^2\) Mutational inactivation of both connexin 47 and connexin 32, and of the potassium channel *KCNJ10*, which localises to oligodendrocytes and astrocytic endfeet (as does CIC-2), also results in myelin vacuolation.\(^7\) Collectively, these proteins are thought to help with the removal of potassium from the clefts between neurons and glia (potassium siphoning). Potassium is taken up into astrocytes to which oligodendrocytes are connected by connexins. Specific deletion of CIC-2 in either cell type is needed to resolve this issue.

The leukodystrophy of *Clcn2\(^{-/-}\)* mice and its similarity to human megalencephalic leukoencephalopathy with subcortical cysts prompted Blanz and colleagues\(^2\) to screen 150 patients with leukoencephalopathy for *CLCN2* mutations. Only a few benign polymorphisms were identified. The same was true in a subsequent screen of 18 patients with megalencephalic leukoencephalopathy with subcortical cysts who did not have *MLC1* mutations.\(^8\) Emphasising the importance of clinical classification, Depienne and colleagues\(^1\) analysed a small cohort with a novel form of leukoencephalopathy. These patients displayed MRI abnormalities in the posterior limbs of the internal capsules, the midbrain cerebral peduncle, or the middle cerebellar peduncle. Symptoms included ataxia and variable spasticity and chorioretinopathy. Besides a mutation in *GJB1*, which encodes connexin 32, the investigators identified six different *CLCN2* mutations in the other patients that were present in either homozygous or compound heterozygous states. Two patients had clear loss-of-function mutations on either allele (predicting truncations within or before the crucial transmembrane part of the channel). Other patients were homozygous for a missense mutation, an in-frame deletion of an intramembrane residue, and a non-sense mutation predicted to delete the large cytoplasmic tail entirely. The latter mutation decreased mRNA levels, and some mutations reduced plasma membrane expression of the protein. Nonetheless, whether the affected proteins retain residual activity that might explain clinical variability, including the age of onset, would be useful to test with electrophysiology. Depienne and colleagues’ immunohistochemical analysis of CIC-2 expression in human brain\(^7\) partly confirmed the knock-out-controlled CIC-2 labelling of mouse brain.\(^2\)
However, the more diffuse labelling, which includes the cytoplasm of axons, for example, might be unspecific. In fact, Westerns blots revealed that the antibodies used strongly crossreacted with other proteins.

No post-mortem brain histopathology was available from any of the patients. Consistent with the myelin vacuolation of Clcn2−/− mice, MRI analysis suggested the presence of white matter microvacuolation. Unlike Clcn2−/− mice, however, all patients presented with ataxia. Whereas Clcn2−/− mice are blind, only some patients had visual impairments, and none was blind. The young age of the only male patient with a CLCN2 mutation precludes a conclusion as to whether this form of leukoencephalopathy is associated with testicular degeneration as in mice. Importantly, none of the patients, nor the Clcn2−/− mice, showed any signs of epilepsy. This observation again supports the conclusion that no evidence exists to suggest that CLCN2 mutations cause epilepsy, a contention largely based on a wrong, now-retracted study.

Another exciting aspect of Depienne and colleagues’ study relates to work showing that GlialCAM, which is based on a wrong, now-retracted study. CLCN2 mutations cause epilepsy, a contention largely based on a wrong, now-retracted study.

Thomas J Jentsch
Leibniz-Institut für Molekulare Pharmakologie (FMP) and Max-Delbrück-Centrum für Molekulare Medizin (MDC), 13125 Berlin, Germany
jentsch@fmp-berlin.de
I declare that I have no conflicts of interest.