No evidence for a role of *CLCN2* variants in idiopathic generalized epilepsy

To the Editor:

We note the retraction of a paper published in Nature Genetics in 2003, which had reported that mutations in *CLCN2* (NCBI Reference Sequence NC_000003.11), the gene encoding the chloride channel ClC-2, were associated with several subtypes of idiopathic generalized epilepsy[1]. Despite the retraction, Kleefuß-Lie *et al.*[2] recently asserted that "other major aspects of the work remain unaltered" and that their electrophysiological studies are supported by further work published subsequently[2]. We believe that their logical argument is flawed and that, in addition, the assertion misrepresents the work of others, including our own.

First, the authors maintain that they "still believe that the reported genetic variations may contribute to the epileptic phenotypes"[2]. Without the link between the reported genetic variations and epilepsy, there is no rational basis for such a belief.

Second, concerning the functional consequences of the mutations, Kleefuß-Lie *et al.*[2] state that "studies in other laboratories… supported some of the functional changes that were originally reported." This statement is untrue. The two papers cited as "studies in other laboratories" come from our respective groups[3,4]. The first of these papers (Niemeyer *et al.*[3]) in fact contradicts every one of the functional findings of Haug *et al.*[1]. The first mutant, 3792_3793insG (M200fsX231), corresponding to family 1 in the retracted publication, predicts a truncated protein lacking 13 out of 18 expected membrane helices including most putative pore-forming regions. The second consists of an 11-bp deletion (2776_2788del11) in intron 2 close to the splice acceptor site, which was suggested to lead preferentially to an alternatively spliced mRNA and a protein, V74_Q117del, lacking most of transmembrane α-helix B, the largest α-helix predicted to lie at the interface between the ClC-2 channel and the membrane. Our results showed that, in contrast to the claims in the retracted paper, these altered proteins did not reach the plasma membrane and did not exert any dominant negative effect on the function of normal ClC-2 (ref. 3). Also, in regard to the 2776_2788del11 mutation, using a minigene approach, we could find no difference in the proportion of exon-skipped to normally spliced mRNA as a consequence of the mutation and, on this basis, predicted no alteration in ClC-2–channel expression in affected individuals. A third mutation, G8794A, produces an amino acid replacement (G715E) purportedly associated with a gain of function[1], allowing the channel to be conductive at reduced intracellular Cl^- concentration. We could not reproduce this result of the retracted paper either[3]. The contrast between our results and those in the retracted paper was reflected in the first paragraph of our Discussion section, which reads: "Our results are in marked contrast to those reported previously by Haug *et al.* and suggest that the pathophysiological mechanisms proposed by these authors to account for the phenotype need to be revised" [3].

The other paper cited as supporting the functional results of the retracted paper is that by Blanz *et al.*[4], in which we in fact confirm the failure to reproduce the dominant negative effect of mutation 3792_3793insG (M200fsX231) reported by Haug *et al.*[1]. We concluded that "our electrophysiological analysis of *CLCN2* sequence abnormalities described in patients with epilepsy (Haug *et al.*) did not provide evidence for them being epileptogenic"[4]. In the same paper[4], we showed that other *CLCN2* sequence variants identified more recently in patients with epilepsy[5] did not alter the biophysical properties of ClC-2 and were also found in humans not displaying epilepsy. We have also reported that the ClC-2–null genotype in mice failed to induce spontaneous seizures or to alter the seizure threshold for the response of the animals to proconvulsants[4,6]. We discussed these points in recent reviews[7,8] and had concluded before the retraction of the paper by Haug *et al.*[1] that "the sum of these observations… warrants skepticism toward the proposed causative role of ClC-2 in epilepsy"[7].

These observations both suggest that even the functional results of the retracted paper cannot be relied upon, and they also support the view that there is no basis to claim that *CLCN2* plays a role in epilepsy.

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