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LETTER TO THE EDITOR

Reply: *ATAD1* encephalopathy and stiff baby syndrome: a recognizable clinical presentation

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Sir,

We welcome the letter by Wolf *et al.* (2018). They report a third family with *ATAD1*-related lethal encephalopathy, further confirming *ATAD1* as a disease gene for the well recognizable stiff baby syndrome. *ATAD1* encodes thorase, an AAA+ ATPase that helps control postsynaptic AMPA receptor internalization by disassembling complexes between glutamate receptor interacting protein (GRIP1) and the AMPA receptor subunit GluA2 (Zhang *et al.*, 2011). The common clinical features shared by all affected members in the three reported families comprise hypertonia, absence of spontaneous movements, almost no motor development, and death within the first months of life (Ahrens-Nicklas *et al.*, 2017; Piard *et al.*, 2018; Wolf *et al.*, 2018). Myoclonic jerks and seizures have been noticed in the affected children reported by Ahrens-Nicklas *et al.* (2017) and the female described by Wolf *et al.* (2018), but in none of the three affected infants we reported (Piard *et al.*, 2018). We agree with Wolf *et al.* (2018) that *ATAD1*-related encephalopathy should be a first-line diagnosis in extremely stiff neonates with or without seizures. However, we are aware of another infantile epileptic encephalopathy, known as rigidity and multifocal seizure syndrome, lethal neonatal (RMFSL; MIM 614498),

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showing an overlapping clinical picture. This phenotype is characterized by intractable seizures, hypertonia, and early death and caused by biallelic mutations in *BRAT1* (Puffenberger *et al.*, 2012; Saunders *et al.*, 2012; Saitsu *et al.*, 2014). We therefore recommend *ATAD1* and *BRAT1* to be preferentially analysed by Sanger-sequencing or in next-generation sequencing panels in the diagnostic work-up of stiff neonates.

The *ATAD1* variant identified by Wolf *et al.* (2018), a homozygous c.162G>C transversion predicting the amino acid substitution p.(Gln54His), has not been tested in the patient's parents to demonstrate that both are heterozygous carriers. Moreover, Wolf *et al.* (2018) did not comment on minor allele frequency or pathogenicity predictions of the *ATAD1* variant. By doing so, we noticed that the c.162G>C change is absent from population databases (ExAC and gnomAD Browsers) and predicted to be damaging by three *in silico* tools combining previous pathogenicity scores [CADD (Kircher *et al.*, 2014), REVEL (Ioannidis *et al.*, 2016), and M-CAP (Jagadeesh *et al.*, 2016)]. As the G-to-C change affects the last nucleotide of exon 2, splicing of the *ATAD1* pre-mRNA has been postulated to be altered (Wolf *et al.*, 2018). We investigated the effect of this sequence change on splicing using four splice site prediction programs: all of them detected the wild-type donor site (http://www.umd.be/HSF3/HSF.shtml; http://www.cbs.dtu.dk/services/NetGene2/; http://www.fruitfly.org/seq_tools/splice.html; http://bioinfo.itb.cnr.it/oriel/splice-view.html). Three recognized the donor site in the mutant sequence with reduced splicing efficiency, while one predicts the complete loss of the donor site. The effect of the *ATAD1* mutation c.162G>C on pre-mRNA splicing and protein function is difficult to predict in the absence of mRNA and functional studies. Nonetheless, it is tempting to speculate that both aberrant *ATAD1* mRNAs, possibly subjected to nonsense-mediated mRNA decay, and mutant ATAD1^{Gln54His} protein had been produced in the affected infant and likely caused (nearly) complete *ATAD1* loss-of-function. Similarly, the homozygous *ATAD1* mutation reported by Ahrens-Nicklas *et al.* (2017) also was a loss-of-function mutation as the c.826G>T change predicted the introduction of a premature termination codon [p.(Glu276*)]. A drastically decreased *ATAD1* mRNA amount and the absence of thorase protein in lymphoblastoid cells derived from one patient further underscored *ATAD1* null alleles in the affected individuals of this family (Ahrens-Nicklas *et al.*, 2017). In contrast, we would like to emphasize that the *ATAD1* mutation c.1070_1071delAT/p.(His357Argfs*15) reported in our family turned out to have a gain-of-function effect, a rarely observed mechanism in autosomal recessively inherited disorders (Piard *et al.*, 2018). Although at first glance the frameshift variant gives the impression of an *ATAD1* loss-of-function mutation, the functional work presented in our study indicated that the encoded thorase mutant^{His357Argfs*15} is expressed in patient cells and gained a novel function of its C-terminal end. We demonstrated decreased levels of surface AMPA receptors in unstimulated mutant^{His357Argfs*15}-expressing neuronal cells compared to wild-type-expressing neurons. We put forward the hypothesis that the thorase mutant^{His357Argfs*15} may inhibit the recycling back and/or reinsertion of AMPA receptors to the surface following endocytosis resulting in a decrease in the steady-state levels of these receptors at the cell surface (Piard *et al.*, 2018). In contrast, biallelic *ATAD1* loss-of-function mutations likely increase the population of excitatory postsynaptic AMPA receptors in neurons as shown in *Atad1*$^{-/-}$ mice (Zhang *et al.*, 2011). In the latter scenario, perampanel, an AMPA receptor antagonist, was shown to decrease tonicity and prevent seizures in two individuals with the homozygous *ATAD1* nonsense mutation p.(Glu276*) (Ahrens-Nicklas *et al.*, 2017). Taken together, these data demonstrate clear benefits of the individualized medicine approach in the two severely affected children reported by Ahrens-Nicklas *et al.* (2017), as also pointed out by Wolf and colleagues (2018). However, we respectfully but strongly disagree with the statement by Wolf *et al.* (2018) to '...ensue a therapeutic trial with perampanel, even before genetic confirmation' in an extremely stiff infant. As both loss- as well as gain-of-function *ATAD1* mutations give rise to the shared neurological features of stiffness and absence of spontaneous movements, we recommend (i) genetic testing in the affected neonates, including *ATAD1* and *BRAT1*, to unravel the genetic basis of the disease; and (ii) determining the consequences of the *ATAD1* mutation, at least on mRNA and protein level in cells obtained from patients. Absence or reduced amount of mutated *ATAD1* transcripts and/or thorase protein in patient-derived cells provide evidence for ATAD1 deficiency, thus opening up the therapeutic potential of perampanel in these patients. On the other hand, therapy with perampanel is not an option in individuals with an *ATAD1* gain-of-function variant, such as the p.(His357Argfs*15) frameshift mutation reported in our study (Piard *et al.*, 2018). Wolf *et al.* (2018) pointed out the importance of detailed clinical work-up for rare neurological disorders and we agree. Beyond that, the work by Ahrens-Nicklas *et al.* (2017) and our work (Piard *et al.*, 2018) highlight the need for determining the molecular basis of rare and devastating neurological disorders and their underlying pathophysiological mechanisms to develop a targeted therapy.

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