LETTER TO THE EDITOR

Reply: ALDH18A1 gene mutations cause dominant spastic paraplegia SPG9: loss of function effect and plausibility of a dominant negative mechanism

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Sir,

In their Letter to the Editor, Panza et al. (2015) share their results following our recent description of mutations in ALDH18A1 in autosomal dominant and recessive hereditary spastic paraplegia (HSP) (Coutelier et al., 2015).

In our report (Coutelier et al., 2015), we used a combination of whole genome mapping, exome sequencing and candidate gene screening, to demonstrate multiple inheritance modes associated with mutations in ALDH18A1 in patients from seven families. Mutations segregating in the autosomal recessive mode had previously been shown to cause De Barsy syndrome (MIM#219150) (Baumgartner et al., 2005). We extended the clinical spectrum to patients without any cutaneous alterations. In addition, we described, for the first time, monoallelic ALDH18A1 mutations segregating in an autosomal dominant way in HSP patients with low levels of plasma ornithine, citrulline, arginine and proline as biological trait biomarkers; this suggests that the pathway involving the enzyme encoded by ALDH18A1 (Δ1-pyrroline-5-carboxylate synthase, P5CS) is deficient. Furthermore, we showed that the enzymatic activity linked to one of the dominant mutations is impaired using indirect measurements of proline synthesis in fibroblasts from two patients.

Panza and colleagues validate our results in autosomal dominant HSP, with two families linked to the SPG9 locus in which heterozygous ALDH18A1 mutations are segregating; one family of British origin segregates the same mutation as found in one of our families. Contrary to the statement by Panza et al. (2015), our manuscript was the first to firmly report dominant cases mutated in ALDH18A1. Indeed, the de novo p.G93R variant reported by Martinelli et al. in a sporadic case (2012) was associated with a p.T299I variant. Even though the latter is present in online databases, its functional effects were demonstrated in vitro in the yeast orthologue by the same authors (Martinelli et al., 2012).

A common clinical picture in both studies (Coutelier et al., 2015; Panza et al., 2015) was the association of spastic paraparesis and congenital cataract, a peculiar association in young patients with autosomal dominant HSP. The presence of gastric signs was only reported in one of
our patients, while it is part of the clinical picture of SPG9. Similarly to two of our families, the six patients from the Italian family from Panza et al. (2015) had significantly low or borderline levels of plasma citrulline, and to a lesser extent reduced plasma ornithine. We believe that the mean of four plasma amino acid levels known to be related to P5CS function (citrulline, ornithine, proline and arginine) and expressed as their age-normalized standard deviations may provide a relevant biomarker as suggested in our study (Coutelier et al., 2015). The observation of low levels of hydroxyproline in two patients by Panza et al. (2015) in the absence of hypoprolinemia is puzzling. Hydroxyproline is mainly produced from proline contained in proteins such as collagen, and this observation might not necessarily be related to P5CS deficiency. However, further investigations are warranted.

In an elegant study that complements our analysis of the global enzymatic activity through measurement of proline synthesis in vitro, Panza et al. provided additional evidence of the pathologial effect of their mutations. They independently explored both enzymatic activities of P5CS in proteins purified from a heterologous overexpression system, and demonstrated nearly complete suppression of both P5CS global activity, and the catalytic activity driven by the L-glutamate 5-kinase domain, in which the mutations are located. As the mutations are at the heterozygous state in patients, the wild-type allele is still expressed; reduced pathway activity could either arise from haploinsufficiency or a dominant negative effect. We pointed out that our data were not conclusive on this issue (Coutelier et al., 2015). Panza et al. showed that the P5CS protein level is diminished in fibroblasts of patients (45% compared to controls), which may be consistent with haploinsufficiency. They brought additional indirect evidence arguing for negative dominance, including a 3D model, and a study of protein hexamer stability. However, a direct test of the effect of the mutated proteins on the wild-type protein activity, either in cells of patients or in an overexpression model, remains to be performed. Thus we cannot exclude that both mechanisms contribute to the mechanism of disease. According to their nature and location, mutation-dependent effects also have to be considered, as pointed out in both manuscripts.

In summary, Panza et al. associate ALDH18A1 mutations to SPG9. Both their and our studies concur to indicate that the P5CS pathway is less efficient due to a possible dominant negative effect of the mutations in autosomal dominant forms. We re-emphasize our proposal to consider the mean of four age-normalized plasma amino acid levels (citrulline, ornithine, proline and arginine) as a biomarker that could be implemented in daily practice in patients with autosomal dominant HSP.

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**References**

