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LETTER TO THE EDITOR

POLR3A variants in hereditary spastic paraplegia and ataxia

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Sir,

We read with great interest the manuscript entitled 'Hypomorphic mutations in *POLR3A* are a frequent cause of sporadic and recessive spastic ataxia', by Minnerop *et al.* (2017). Based on our own clinical expertise on POLR3-related disorders, as well as data from 745 individuals from 492 families with hereditary spastic paraplegia (HSP) and ataxia, we would like to raise one main concern. Based on the recent literature, we believe that the patients described by Minnerop *et al.* would have been better categorized as having POLR3-related disorders, rather than ataxia or HSP. Moreover, our genetic data suggest that the intronic variant in *POLR3A* (c.1909+22G>A) is not likely to cause HSP and ataxia, but rather, atypical POLR3-related disorders as previously reported (La Piana *et al.*, 2016). Ultimately, functional studies looking at the impact of the c.1909+22G>A intronic variant will shed light on its exact role.

The clinical characteristics of POLR3-related disorders have been described as an overlapping phenotype with features of both ataxia and HSP since the earliest descriptions (Wolf *et al.*, 2005; Timmons *et al.*, 2006; Bernard *et al.*, 2010). Indeed, patients with POLR3-related disorders have prominent cerebellar features, including gait ataxia, intention tremor, dysmetria and saccadic pursuit. Pyramidal features are typically less prominent, and involve more the lower than the upper extremities. We would like to emphasize that peripheral neuropathy is not a feature of

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POLR3-related disorders, as nerve conduction studies (NCS) are normal in all cases our group is aware of. Moreover, the pathological findings described on electron microscopy by Timmons *et al.* (2006) are hypothesized to be most likely artefactual, as they have never been replicated. Therefore, we wonder whether the abnormal NCS reported in six patients (Patients F1-5, F1-8, F3-1, F11-1, F12-1 and F19-1) by Minnerop *et al.* (2017) are caused by another aetiology.

Non-neurological features are not obligatory in POLR3-related disorders and include a wide range of dental abnormalities and endocrine manifestations (typically hypogonadotropic hypogonadism, i.e. pituitary dysfunction rather than gonadal dysfunction). Myopia was not mentioned by Minnerop *et al.*, however, it is the most common non-neurological feature. Another frequent non-neurological manifestation in POLR3-related disorders is short stature, which is seen in approximately half of the patients (Wolf *et al.*, 2014). It would have been interesting to know the height of the patients presented by Minnerop *et al.* Regarding the dental abnormalities, the authors reported a frequency of 65% (11 of 17 cases when asked specifically); however, they did not have the information on 9/29 patients, therefore the frequency of this manifestation in their cohort is not clear. In the largest cohort of POLR3-related leukodystrophy patients published thus far, the frequency of dental abnormalities was significantly higher (87%), and the abnormalities could be very subtle (Wolf *et al.*, 2014). Therefore, we believe that it is useful to routinely add questions about dentition and teeth to the review of systems in patients with neurodegenerative disorders, as dental abnormalities are often not spontaneously reported. Interestingly, seven patients in the cohort of Minnerop *et al.* had a detailed dental evaluation (Patients F1-3, F1-5, F1-7, F1-8, F2-1, F3-1 and F14-1) and all of them were found to have mild abnormalities, possibly suggesting a higher frequency than 65%.

In POLR3-related disorders, brain MRI typically shows hypomyelination with better myelination of specific structures (i.e. dentate nucleus, optic radiation, anterolateral nucleus of the thalamus, globus pallidus, corticospinal tracts at the level of the posterior limb of the internal capsule), with or without thinning of the corpus callosum and atrophy of the cerebellum (Steenweg *et al.*, 2010; La Piana *et al.*, 2014; Wolf *et al.*, 2014). Patients with hypomyelination and absence of these other MRI features are unlikely to have mutations in *POLR3A* or *POLR3B* (Cayami *et al.*, 2015). In our opinion, this observation still holds true. Some patients have atypical MRI patterns without hypomyelination, as described by Minnerop *et al.* and as previously reported (La Piana *et al.*, 2016). Of note, however, Minnerop *et al.* reported complete brain MRI results for only 12/29 patients in their study. Therefore, it may be premature to determine that their cohort does not exhibit the typical white matter features of POLR3-related disorders. Furthermore, the MRI images provided (Fig. 4) are incomplete and it is difficult to examine the presence or absence of certain previously reported MRI characteristics. Figure 4, B1 (from Minnerop *et al.*, 2017) shows mild superior cerebellar vermis atrophy (that should be confirmed on coronal views) and mild thinning of the corpus callosum without hypomyelination. This pattern has already been described by La Piana *et al.* (2016). Regarding the MRI abnormalities involving the superior cerebellar peduncles, we thank the authors for this observation. We went back to our atypical cases published in 2016 and found 4/8 cases with this same finding. We therefore suggest that this MRI characteristic is not mutation-specific as the authors hypothesized, but rather is part of the MRI pattern seen in atypical POLR3-related disorders. Regarding the homozygous c.1771-7C>G variant, it would have been useful to have the complete MRI features of these patients. This mutation seems to be associated with a very different clinical and, we would assume, MRI phenotype, which appears to be similar to what has been previously reported (Azmanov *et al.*, 2016). Finally, we believe that it is extremely important to review the brain MRI of all patients with ataxia and HSP because the presence of white matter abnormalities can be a major clue to the diagnosis and lead to a significant change in classification of the suspected diseases.

In addition to these clinical considerations, our genetic data suggest that the c.1909+22G>A variant (rs191875469) is unlikely to be a major cause of HSP and ataxia. Our group first reported this variant in 2016; however, its pathogenicity could not be fully resolved at the time as not enough material was available to perform the necessary experiments (La Piana *et al.*, 2016). With the work by Minnerop *et al.* (2017), it now appears clear that the c.1909+22G>A variant is indeed pathogenic. However, our genetic data do not support a role for this variant in large cohorts of HSP and ataxia. We examined exome-sequencing data from 745 individuals from 492 families with HSP and ataxia. The average coverage of *POLR3A* across all samples was >100×, with 100% of the nucleotides covered with >10×. Among 257 families with HSP, the c.1909+22G>A variant was identified in three families. However, in none of them it seemed to be disease-related. One large family had an autosomal dominant pattern of inheritance, which did not fit the recessive model of *POLR3A*-related disorders. Furthermore, the c.1909+22G>A variant did not segregate with the eight affected individuals that were sequenced in this family. In two other families with only one patient in each, the two patients were heterozygous carriers of the c.1909+22G>A variant but did not carry any other *POLR3A* mutation. However, we still cannot rule out structural variants or large copy number variations in *POLR3A* that cannot be detected in exome sequencing. In 135 families with ataxia, which included 170 individuals, the c.1909+22G>A variant was not identified at all. The allele frequency of this variant in our index cases was 0.33%, very similar to the control frequencies reported by Minnerop *et al.* (2017) (0.38% and 0.35%).

In conclusion, we would like to reiterate the importance of classifying patients with neurodegenerative disorders by combining fine clinical phenotyping and MRI features, as it can be of great help to target the investigations. Regarding the cohort reported by Minnerop *et al.* (2017), we would first recommend a thorough expert review of the brain MRI of all patients. We would hypothesize that several, if not all cases, would probably be categorized as POLR3-related disorders rather than HSP or ataxia. The genetic evaluation of the potentially non-POLR3-related cases (if any) could be completed by whole exome or genome sequencing to identify another genetic aetiology. We would also suggest looking for large deletions or duplications in patients with a compatible phenotype to find any other disease-causing variant.

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