doi:10.1093/brain/awx291 BRAIN 2018: 141; 1–4 | e2

BRAIN
A JOURNAL OF NEUROLOGY

LETTER TO THE EDITOR

Reply: *POLR3A* variants in hereditary spastic paraplegia and ataxia

Martina Minnerop,[1,2] Delia Kurzwelly,[2,3] Tim W. Rattay,[4,5] Dagmar Timmann,[6] Holger Hengel,[4,5] Matthis Synofzik,[4,5] Claudia Stendel,[7,8] Rita Horvath,[9] Rebecca Schüle[4,5] and Alfredo Ramirez[10,11,12,13]

1 Institute of Neuroscience and Medicine (INM-1), Research Centre Juelich, 52425 Jülich, Germany
2 Department of Neurology, University of Bonn, 53127 Bonn, Germany
3 German Center for Neurodegenerative Diseases (DZNE), 53127 Bonn, Germany
4 Center for Neurology and Hertie Institute for Clinical Brain Research, University of Tübingen, 72076 Tübingen, Germany
5 German Center for Neurodegenerative Diseases (DZNE), 72076 Tübingen, Germany
6 Department of Neurology, University of Duisburg-Essen, 45147 Essen, Germany
7 Department of Neurology, Friedrich-Baur-Institute, Ludwig-Maximilians-Universität, 80336 Munich, Germany
8 German Center for Neurodegenerative Diseases (DZNE), 81337 Munich, Germany
9 Wellcome Trust Centre for Mitochondrial Research, Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne NE1 3BZ, UK
10 Department of Psychiatry and Psychotherapy, University of Bonn, 53127 Bonn, Germany
11 Institute of Human Genetics, University of Bonn, 53127 Bonn, Germany
12 Department of Psychiatry and Psychotherapy, University of Cologne, 50937 Cologne, Germany
13 Clinic for Neurodegenerative diseases and geriatric psychiatry, University of Bonn, 53127 Bonn, Germany

Correspondence to: Alfredo Ramirez,
Department of Psychiatry and Psychotherapy, University of Cologne, Kerpener Strasse 62, 50937 Cologne, Germany
E-mail: alfredo.ramirez@uk-koeln.de

Sir,

We thank Gauquelin *et al.* (2017) for their interesting letter regarding our recent publication 'Hypomorphic mutations in *POLR3A* are a frequent cause of sporadic and recessive spastic ataxia' (Minnerop *et al.*, 2017). The authors of the letter question the pathogenic relevance of the novel hypomorphic intronic *POLR3A* variant, which we observed in a total of 19 independent families. Gauquelin *et al.* suspected undetected mutations in other HSP and ataxia genes in at least a subset of our families, while at the same time arguing that the phenotype we reported in those very same families falls well within the spectrum of POLR3-related disease and should rather be classified as atypical POLR3-related disease. They put forth clinical as well as genetic arguments to support these somewhat contradictory statements.

Gauquelin *et al.* questioned whether the reported peripheral neuropathy in some of our cases can truly be attributed to the *POLR3A* gene-related defect and speculated about a possible different aetiology. While alternative causes cannot ultimately be ruled out, they are highly unlikely given that (i) diagnostic work-up did not provide evidence for secondary causes of neuropathy in any of the cases; and that (ii) neuropathy was present even in childhood-onset cases where secondary causes of neuropathy are rare. Presence of childhood-onset neuropathy in Patient F19-1 carrying the c.1909+22G>A mutation at homozygous state further supports neuropathy as a POLR3-related clinical feature at least in carriers of that specific hypomorphic variant. Lastly, (iii) the frequent occurrence of neuropathy in our cohort (5/17, 29% of cases) render it unlikely that it is just a coincidental finding secondary to other causes.

We thank the authors for pointing out myopia and short stature as common non-neurological features in POLR3-related disorders, both of which were not directly obvious in our patients. We now aggregated more systematic information on these features (Table 1). Myopia was only present in 8/20 patients carrying the c.1909+22G>A mutation in a compound heterozygous state and neither particularly pronounced or progressive as reported by

Advance Access publication December 11, 2017

Table 1 Myopia and body height in *POLR3A* patients

Patient ID	c.1909+22G>A	Second mutation	Mode of inheritance/gender	Race/origin	Age at onset (y)	Age at exam (y)	Myopia	Body height (cm)
F1-3	Het	Q31*	AR/F	CAU/GER	51	57	No	156
F1-5	Het	Q31*	AR/M	CAU/GER	15	53	Yes	170
F1-7	Het	Q31*	AR/F	CAU/GER	20	50	No	165
F1-8	Het	Q31*	AR/M	CAU/GER	14	48	No	172
F2-1	Het	D372N	AR/F	CAU/GER	20	47	2 y in adolescence	172
F3-1	Het	F431Sfs*26	S/M	CAU/GER	15	68	No	180
F4-1	Het	E1261K	S/M	CAU/GER	20	56	No	170
F5-1	Het	L454F	S/M	CAU/GER	28	38	No	165[b]
F6-1	Het	S825Qfs*18	S/M	CAU/GER	13	27	No	170
F7-1	Het	G904*	AR/F	CAU/USA	17	30	No	173
F8-1	Het	A515V	S/M	CAU/UK	18	31	Yes	193
F9-1	Het	G854Afs*5	S/M	CAU/GER	12	42	Yes	173
F10-1	Het	Splice	S/F	CAU/GER	26	56	Yes	169
F11-1	Het	Q511*	S/M	CAU/GER	12	66	No	176
F12-1	Het	V1315fs*7	S/M	CAU/GER	18	41	Yes	182
F13-1	Het	K713Kfs*3	S/F	CAU/GER	6	50	Yes	165[a]
F14-1	Het	C109S	S/F	CAU/GER	12	45	No	163
F15-1	Het	G350Gfs*27	AR/F	CAU/GER	31	45	No	165
F16-1	Het	R873*	AR/F	CAU/UK	21	30	Yes	147[a,b]
F16-2	Het	R873*	AR/F	CAU/UK	23	35	N/E	N/E
F17-1	Het	M852fs*7	S/M	CAU/UK	12	25	N/E	N/E
F18-1	Het	L356P	S/M	CAU/UK	20	42	Yes	155[a,b]
F19-1	Hom	-	S/F	CAU/USA	11	18	N/E	N/E
F20-1	-	V1033A (hom)	AR/M	CAU/BEL	2	20	No	169
F21-3	-	c.1771-7C>G (hom)	S/M	CAU/GER	16	55	No	Normal[c]
F22-6	-	c.1771-7C>G (hom)	AR/F	CAU/TR	0	29	No	163
F22-7	-	c.1771-7C>G (hom)	AR/F	CAU/TR	0	21	No	163
F23-3	-	c.1771-7C>G (hom)	AR/M	CAU/IL	2	12	N/E	Short[c]
F23-5	-	c.1771-7C>G (hom)	AR/F	CAU/IL	2	19	N/E	Short[c]

AR = autosomal recessive; BEL = Belgium; CAU = Caucasian; F = female; GER = Germany; Het = heterozygous; Hom = homozygous; IL = Israel; M = male; N/E = not evaluated; S = sporadic; TR = Turkey.

[a]Short stature according absolute body height values (males <166 cm, females <153 cm).

[b]Short stature in relation to parental body height.

[c]According to the subjective assessment of the clinician in charge.

Wolf *et al.* (2014). In contrast to our patients, their patient sample included patients with mutation in either *POLR3A* or *POLR3B* and most patients had a disease onset before the age of 10 years, while our patients developed their first neurological symptoms at a median age of 18 years. These differences further emphasize that we are dealing with different phenotypes and myopia does not seem to be a prominent feature in our patient sample, at least not with pronounced incapacitating impairments on patients' daily lives. With respect to short stature as a further non-neurological feature, two out of five patients carrying the homozygous c.1771-7C>G mutation were reported to have short stature (both belonging to the same family), while only three (two males) of the 20 patients carrying the c.1909+22G>A mutation in a compound heterozygous state (height information was lacking in further two) fulfilled the definition of short stature (males <166 cm, females <153 cm). However, body height is influenced by different factors such as ethnic background, parental height, socioeconomic status, year of birth, etc. Unfortunately, neither Wolf *et al.* (2014) nor La Piana *et al.* (2016) provided a definition of the statement 'short stature' they used, and the information regarding the precise height of their patients is also missing. If the parental height was taken into account (available in 16 patients), only three patients (one male) were below the 95% target range of expected body height (Falkner and Tanner, 1986). In conclusion, short stature can hardly be regarded as a prominent non-neurological feature in our patient sample.

We agree with Gauquelin *et al.* that we may underestimate the frequency of dental abnormalities in our cohort as the frequency we report in the larger cohort (11/17, 65%) is based on patient history, although patients were specifically prompted to report any dental issues, while the frequency we found in patients that received a dedicated dental examination was indeed higher (all seven patients examined). Due to the international multicentre setting of our study, detailed dental work-up for all patients was unfortunately not feasible. The important recommendation by the authors to include questions regarding dentition in the diagnostic work-up of patients with neurodegenerative movement disorders is however highly appreciated.

We agree with the authors that the presence of typical white matter features of POLR3-related disorders in cases without comprehensive MRI results cannot be excluded. However, myelination was possible to evaluate in most patients (20/29 patients; Minnerop *et al.*, 2017). Additional POLR3-typical MRI features including cerebellar atrophy (1/22) or thinning of the corpus callosum (7/16) were infrequently present in those cases with comprehensive MRI scans. We agree with the authors that there is some overlap between the MRI pattern described by La Piana *et al.* (2016) and the pattern observed in our patients. However, the most distinguishing MRI features in our cohort were the bilateral hyperintensities along the superior cerebellar peduncles on FLAIR images observed in 11/12 patients with comprehensive MRI scans, which have not been described in POLR3-related disorders before. It is

therefore of great interest that Gauquelin *et al.* confirmed on re-evaluation the same finding in 4/8 of their previously described POLR3-related cases with atypical MRI pattern (La Piana *et al.*, 2016). In light of these new results, the cerebellar peduncular abnormalities may indeed not be specific for the 1909+22G>A mutation but may represent a previously unreported finding in atypical POLR3-related disorders. However, it would have been helpful to know whether any of their four cases carried the c.1909+22G>A variant (rs191875469) and whether these cases also included *POLR3B* mutation carriers. We agree with the authors that comprehensive MRI scans would have been of special interest in those cases carrying the homozygous c1771-7C>G variant and actually presenting a different clinical phenotype. The evaluation of brain MRI in all patients presenting with an ataxia or HSP phenotype, in particular in combination with questions regarding dentition, can therefore indeed provide significant clues towards the diagnosis and will hopefully help to uncover previously undiagnosed POLR3-related disorders.

The findings presented by Minnerop *et al.* (2017) are the first large screening of *POLR3A* mutations in phenotypes without any overt signs of hypomyelinating leukodystrophy. As such, the estimated odds ratio (OR) of the association with c.1909+22G>A may suffer the so-called 'winner's curse', i.e. OR of true association is inflated due to a given threshold for statistical significance combined with an underpowered sample to perform the discovery with this variant (Ioannidis, 2008; Kraft, 2008). For this reason, the findings of Minnerop *et al.* (2017) need confirmation in independent samples. The results presented by Gauquelin *et al.* are the first replication effort in an independent sample of HSP and ataxia phenotypes. This analysis included exome-sequencing data from 745 individuals from 492 families with either HSP or ataxia. While the authors identified three heterozygous carriers of c.1909+22G>A only in HSP families, none of the ataxia families showed this variant. Furthermore, the authors found an allele frequency for the rare allele of c.1909+22G>A of 0.33% in their sample, very similar to the frequency reported in the control sample of Minnerop *et al.* (2017). Based on these findings, Gauquelin *et al.* concluded that c.1909+22G>A is not a frequent cause of HSP or ataxia. However, the results presented by Gauquelin *et al.* should be interpreted with caution, as they lack several criteria to formally consider their study a replication study including similar sample selection, study design, and statistical method applied for replication (Kraft *et al.*, 2009).

Firstly, both studies differ in design and sample selection. Minnerop *et al.* (2017) started from one unsolved family classified as recessive HSP in which the authors performed classical linkage analysis followed by whole exome sequencing. The initial findings led the authors to search in additional unexplained sporadic and recessive cases with ataxia, spastic paraplegia, and peripheral neuropathy. Hence, the study design in Minnerop *et al.* lack *a priori* hypothesis on the causative gene, i.e. *POLR3A*. In contrast, the authors in Gauquelin *et al.* have longstanding research experience on the genetics of *POLR3A*, which may have introduced bias in the sample used in this study (Daoud *et al.*, 2013; Wolf *et al.*, 2014; Cayami *et al.*, 2015). In fact, as stated by Gauquelin *et al.*, the author described previously c.1909+22G>A in a patient presenting ataxia and hypodontia or dental abnormality (La Piana *et al.*, 2016). Thus, the clinical awareness of the authors regarding features related to *POLR3A* mutations may have produced a systematic depletion of potential *POLR3A* mutation carriers in both the HSP and the ataxia cohorts. This bias introduced in their 492 families may have led to the identification of three heterozygous carriers of c.1909+22G>A only in the HSP families. Furthermore, the same reason may explain the 0.33% allele frequency for the rare allele of c.1909+22G>A reported now by Gauquelin *et al.* Hence, the sample studied by Gauquelin *et al.* resembles more the 'disease controls' cohort used by Minnerop *et al.*, which included neurological disorders and features not related to the *POLR3A* spectrum.

Secondly, Gauquelin *et al.* did not include a proper control sample to perform the statistical analysis, a critical step for a formal replication study. Importantly, several genetic studies on rare variants, such as c.1909+22G>A, have clearly shown differences in the allele frequency for the same variant within European countries, i.e. Caucasian population (Benitez *et al.*, 2013; Cruchaga *et al.*, 2014; Genome of the Netherlands, 2014; Ruiz *et al.*, 2014; Heilmann *et al.*, 2015). Minnerop *et al.* (2017) presented genotype data for c.1909+22G>A in one 'disease controls' sample originating from the same database as the patient samples and one healthy control sample from Germany matching the ethnic background of several patients in Minnerop *et al.* (2017). Following this line of arguments, the allele frequency reported for c.1909+22G>A in ExAC showed some differences across populations, i.e. in European [minor allele frequency (MAF) = 0.0019], in South Asian (MAF = 0.0008), in Latino (MAF = 0.0009), and in African (MAF = 0.0004) populations. Interestingly, all populations, including the European population, have MAF for c.1909+22G>A lower than the 0.33% (0.0033) reported by Gauquelin *et al.* In fact, the minor adenine allele of c.1909+22G>A is significantly enriched ($P = 3.8 \times 10^{-3}$, OR = 4.64, confidence interval 95% = 1.48–14.56) when comparing the allele frequency reported by Gauquelin *et al.*, based on three carriers in 492 independent families, with that reported in ExAC. Hence, Gauquelin *et al.* would have formally replicated the association reported by Minnerop *et al.* (2017). Unfortunately, it should be noted that Gauquelin *et al.* did not present clearly how they obtained the allele frequency for c.1909+22G>A. In fact, the authors stated that the frequency of this variant in their index cases is 0.33%. However, it is unclear whether these index cases refer to one case from each of the 492 independent families or to the 100 cases left after subtracting 257 HSP families and 135 ataxia families ($n = 392$). Finally, Gauquelin *et al.* present results based on the screening of 492 independent

cases that is definitely underpowered to confirm or refute the association presented by Minnerop *et al*. The 492 index cases have a power of 13% to detect an association considering a high risk allele frequency of 0.0033, a prevalence for the disease of 0.0005, an OR for heterozygous carriers of 1.69 (lower value for the confidence interval in Minnerop *et al*., 2017).

The lack of segregation of c.1909+22G>A reported by Gauquelin *et al*. in their three HSP families is not surprising because the mode of inheritance reported for *POLR3A* mutation is recessive, i.e. both alleles must be affected. If no additional mutations in *POLR3A* are observed in these families, it cannot be expected that c.1909+22G>A segregates with the phenotype as this mutation is not sufficient to cause the disease, and therefore mutations in other genes may explain the phenotype in these families. Nevertheless, it would be interesting to study the phenotype of those patients carrying c.1909+22G>A as this variant may act as modifier of the phenotype produced by mutations in other genes.

Gauquelin *et al*. emphasize the importance of searching for larger duplications or deletions in *POLR3A* as potential missing cause. However, the authors have published several screenings of *POLR3A* in different cohorts and never found evidence suggesting that deletions or duplications might be relevant potential pathogenic mechanisms in *POLR3A* driven phenotypes. It is therefore unlikely now that *POLR3A* duplications and deletions explain all patients carrying c.1909+22G>A reported by Minnerop *et al*. (2017). Moreover, the majority of patients described by Minnerop *et al*. (350 of 355 patients screened, 99%) were screened with next generation sequencing technology covering the entire exome or at least the genes linked to *POLR3A*-related phenotypes, including HSP and ataxia (see Supplementary material in Minnerop *et al*., 2017). Hence, it is again unlikely, as stated by Gauquelin *et al*., that patients studied by Minnerop *et al*. are caused by mutations in other genes. Noteably, c.1909+22G>A segregated with the phenotype in two families reported by Minnerop *et al*. (2017) where DNA was available to perform the segregation analysis.

In conclusion, Gauquelin *et al*. present findings that do not support the results and conclusion reported by Minnerop *et al*. However, several caveats in their study design render results of Gauquelin *et al*. difficult to interpret casting doubt on their conclusions. From a genetic perspective, the study is underpowered and key elements for a formal replication study are missing.

Funding

Research reported in this publication was supported in part by the National Institute of Neurological Disorders and Stroke and the National Institutes of Health under Award Number 5R01NS072248. In addition, support for this project was received from the European Union's Horizon 2020 research and innovation program under the ERA-NET cofund action N° 643578 in the framework of the E-Rare-3 network PREPARE (BMBF, 01GM1607 to M.S.; associated PREPARE network members R.H.). T.W.R. receives funding from the University of Tübingen, medical faculty, for the Clinicians Scientist Program grant:386-0-0. Additional support was obtained from the German Research foundation (DFG) trilateral project (reference number SCHO 754/5-2).

References

Benitez BA, Cooper B, Pastor P, Jin SC, Lorenzo E, Cervantes S, et al. TREM2 is associated with the risk of Alzheimer's disease in Spanish population. Neurobiol Aging 2013; 34: 1711.e15–7.

Cayami FK, La Piana R, van Spaendonk RM, Nickel M, Bley A, Guerrero K, et al. POLR3A and POLR3B mutations in unclassified hypomyelination. Neuropediatrics 2015; 46: 221–8.

Cruchaga C, Karch CM, Jin SC, Benitez BA, Cai Y, Guerreiro R, et al. Rare coding variants in the phospholipase D3 gene confer risk for Alzheimer's disease. Nature 2014; 505: 550–4.

Daoud H, Tetreault M, Gibson W, Guerrero K, Cohen A, Gburek-Augustat J, et al. Mutations in POLR3A and POLR3B are a major cause of hypomyelinating leukodystrophies with or without dental abnormalities and/or hypogonadotropic hypogonadism. J Med Genet 2013; 50: 194–7.

Falkner FT, Tanner JM. Human growth: a comprehensive treatise. New York: Plenum Press; 1986.

Gauquelin L, Tetreault M, Thiffault I, Farrow E, Miller N, Yoo B, et al. POLR3A variants in hereditary spastic paraplegia and ataxia. Brain 2017; 141: e1.

Genome of the Netherlands Consortium. Whole-genome sequence variation, population structure and demographic history of the Dutch population. Nat Genet 2014; 46: 818–25.

Heilmann S, Drichel D, Clarimon J, Fernandez V, Lacour A, Wagner H, et al. PLD3 in non-familial Alzheimer's disease. Nature 2015; 520: E3–5.

Ioannidis JP. Why most discovered true associations are inflated. Epidemiology 2008; 19: 640–8.

Kraft P. Curses-winner's and otherwise—in genetic epidemiology. Epidemiology 2008; 19: 649–51; discussion 57–8.

Kraft P, Zeggini E, Ioannidis JP. Replication in genome-wide association studies. Stat Sci 2009; 24: 561–73.

La Piana R, Cayami FK, Tran LT, Guerrero K, van Spaendonk R, Ounap K, et al. Diffuse hypomyelination is not obligate for POLR3-related disorders. Neurology 2016; 86: 1622–6.

Minnerop M, Kurzwelly D, Wagner H, Soehn AS, Reichbauer J, Tao F, et al. Hypomorphic mutations in POLR3A are a frequent cause of sporadic and recessive spastic ataxia. Brain 2017; 140: 1561–78.

Ruiz A, Dols-Icardo O, Bullido MJ, Pastor P, Rodriguez-Rodriguez E, Lopez de Munain A, et al. Assessing the role of the TREM2 p.R47H variant as a risk factor for Alzheimer's disease and frontotemporal dementia. Neurobiol Aging 2014; 35: 444.e1–4.

Wolf NI, Vanderver A, van Spaendonk RM, Schiffmann R, Brais B, Bugiani M, et al. Clinical spectrum of 4H leukodystrophy caused by POLR3A and POLR3B mutations. Neurology 2014; 83: 1898–905.