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## Short Report

# Clinical and *in silico* evidence for and against pathogenicity of 11 new mutations in the *MPZ* gene

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Mutations in the myelin protein zero (*MPZ*) gene are one of the frequent causes of Charcot-Marie-Tooth (CMT) hereditary neuropathies. Because the mutation rate of *MPZ* gene is rather high and some mutations are reported as polymorphisms, the proper clinical, electrophysiological examination and the segregation of the new mutation in larger families are crucial for the correct interpretation of the pathogenic or non-pathogenic character of each novel mutation. We examined 11 families with novel *MPZ* mutations. Eight of the mutations (L48Q, T65N, E97fs, G103W, P132T, T143R, V146G, c.645+1G>T) seem to be pathogenic on the basis of perfect segregation with the CMT phenotype and two (G213R and D246N), on the contrary, seem to be non-pathogenic/rare polymorphisms because they are present in healthy relatives. The character of the V46M mutation is difficult to interpret definitely; it may cause a sensory neuropathy or may also be a rare polymorphism. Phenotypes associated with each of the new mutations include severe hereditary motor and sensory neuropathy type III (HMSN III), and mild phenotype CMT1B presented mostly with only decreased or absent reflexes, foot deformities and mild or even absent atrophies in the lower limbs. Our report and careful family investigations with genotype–phenotype correlations should help to improve genetic counselling and correct interpretation of DNA testing results in further isolated patients or smaller families worldwide where these novel mutations might be found.

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Patients with hereditary neuropathy Charcot-Marie-Tooth (CMT) suffer from distal muscle weakness, atrophies and abnormalities of the tendon reflexes earlier and more pronounced in the lower limbs than in the upper limbs.

Mutations in the myelin protein zero (*MPZ*) gene coding for the P0 myelin protein are one of the most frequent causes of CMT and are associated with four different phenotypes (1, 2); (i) CMT1B, the demyelinating neuropathy very similar to CMT1A, with decreased nerve conduction velocities (NCVs) below 38 m/s (3), (ii) Dejerine-Sottas neuropathy (DSN) – also called hereditary motor and sensory neuropathy type III (HMSN III) – an infantile onset neuropathy often with delayed early motor milestones and severe affection of myelinization with extremely decreased NCVs, (iii) CHN – congenital hypomyelinating neuropathy with congenital onset, severe handicap and frequently shortened life expectancy and (iv) CMT2 – the late-onset axonal neuropathy, with decreased compound muscle action potential (CMAP) amplitudes and normal or nearly normal NCV (4–6).

More than 120 pathogenic mutations, mostly point mutations, have been reported and are

listed in the CMT mutation database (http://www.molgen.ua.ac.be/CMTMutations/). Only three reported intronic pathogenic mutations listed in this database are predicted to affect the protein by altering the splice recognition site (7–9). *MPZ* has a high mutation rate; *de novo* mutations are frequently the cause of HMSN III in sporadic patients and several mutations turned out to be non-pathogenic polymorphisms. Therefore, the interpretation of a finding of a novel mutation is difficult or impossible in isolated patients. Segregation analysis in larger families and detailed neurological, electrophysiological and DNA examination are therefore crucial for the correct interpretation of the pathogenicity of each novel mutation.

We report the phenotypic expression of 11 new *MPZ* sequence variants found in Czech CMT patients and provide clinical, electrophysiological and *in silico* evidence for and against the pathogenicity of these novel mutations.

## Materials and methods

The new MPZ sequence variants were detected during routine DNA testing for CMT in our laboratory. The inclusion criteria for *MPZ* gene testing reflected the four phenotypes known to be associated with *MPZ* mutations (CMT1B, HMSN III, CMT2-late onset, CHN). All patients were previously tested negative for the most frequent type of CMT – CMT1A duplication/hereditary neuropathy with liability to pressure palsies (HNPP) deletion using the set of 17 microsatellite markers (10). All patients have signed informed consent with the analysis of hereditary neuropathy-related genes.

The six coding exons of the *MPZ* gene were amplified in four polymerase chain reaction (PCR) reactions using a Plain PP Master Mix from Top-Bio, Prague, Czech Republic (www.top-bio.cz). PCR primers were used as described by Nelis et al. (11). Direct sequencing was performed on an ABI3100 Avant Genetic Analyzer (Applied Biosystems, Foster City, CA).

For historical reasons, the names of the mutations are based on the *MPZ* sequence D10537, where the initiation codon is 30 nucleotides downstream and the protein sequence is 10 amino acids (AAs) shorter than the reference protein sequence NM_000530.

Computer analysis for the probability of a deleterious effect on the protein function was made by the program PANTHER (http://www.pantherdb.org) (12, 13). PANTHER calculates the substitution position-specific evolutionary conservation (subPSEC) score based on a multiple sequence alignment of evolutionary related proteins. The subPSEC scores below −3 from the PANTHER refer to the disease-causing mutations (13). We used the reference sequence NP_000521.

## Results

We present phenotypes associated with 11 new *MPZ* sequence variants: p.Val46Met, p.Leu48Gln, p.Thr65Asn, p.Glu97fs, p.Gly103Trp, p.Pro132Thr, p.Thr143Arg, p.Val146Gly, c.645+1G>T, p.Gly213Arg and p.Asp246Asn. In addition, we found a silent mutation p.Ala15Ala in the first exon of the *MPZ*. An overview of the sequence variants is presented in Table 1. None of the 11 new sequence variants was found in 188 control healthy chromosomes from unrelated and anonymous control subjects.

Variants p.Gly213Arg and p.Asp246Asn seem to be non-pathogenic as they are present in healthy relatives (Fig. 1, families 11 and 12). The remaining eight mutations we interpret as pathogenic because they segregate perfectly with the CMT phenotype in the families, or are *de novo* as in all the HMSN III families. For the p.Val46Met, the pathogenicity could not be reliably clarified; it may represent a hypomorphic allele causing a late-onset mild neuropathy or it may represent a rare non-pathogenic polymorphism. The patient suffered mostly from sensory neuropathy with no muscle weakness.

One mutation, p.Leu48Gln, was found in two families. We assume that these families must be related as they live in the same region, but we cannot prove it because patient 2 grew up without contact with his parents.

The clinical findings in patients with pathogenic mutations are summarized in Table 2.

Three mutations – p.Thr65Asn, p.Gly103Trp and p.Pro132Thr – cause the severe HMSN III phenotype with onset in infancy. The patients did not walk independently until after 18 months and they have unstable walking even as adults. We found scoliosis in all but one of these HMSN III patients, the younger patient 7 (2 years of age) being the exception. The NCVs were severely decreased in all these HMSN III patients (Table S1). All mutations with HMSN III phenotype were *de novo* mutations confirmed by DNA testing (families 4 and 7) or by family history (family 6). The paternity testing was performed in patient 4, in other families the father was not alive or refused the paternity testing.

Five mutations – p.Leu48Gln, p.Glu97fs, p.Thr 143Arg, p.Val146Gly and c.645+1G>T – were

Table 1. New pathogenic and non-pathogenic myelin protein zero (*MPZ*) mutations in Czech Charcot-Marie-Tooth (CMT) patients

| Nucleotide position | Codon change | Aminoacidic change | Protein domain | Family | Family history | PANTHER-SubPSEC | Mutation character |
|---|---|---|---|---|---|---|---|
| c.136G>A | GTG-ATG | p.Val46Met | EC (Ig-like) | 1 | Familiar (2) | −4.27719 | Unresolved |
| c.143T>A | CTG-CAG | p.Leu48Gln | EC (Ig-like) | 2 | Familiar (3) | −7.24343 | Causal–pathogenic |
| c.143T>A | CTG-CAG | p.Leu48Gln | EC (Ig-like) | 3 | Familiar (7) | −7.24343 | Causal–pathogenic |
| c.194C>A | ACC-AAC | p.Thr65Asn | EC (Ig-like) | 4 | *De novo* (1) | −2.34049 | Causal–pathogenic |
| c.289_292delGAGC | — | p.Glu97fs | EC (Ig-like) | 5 | Familiar (4) | — | Causal–pathogenic |
| c.307G>T | GGG-TGG | p.Gly103Trp | EC (Ig-like) | 6 | *De novo* (2) | −8.33821 | Causal–pathogenic |
| c.394C>A | CCT-ACT | p.Pro132Thr | EC (Ig-like) | 7 | *De novo* (1) | −6.21197 | Causal–pathogenic |
| c.428C>G | ACG-AGG | p.Thr143Arg | EC (Ig-like) | 8 | Familiar (3) | −1.99378 | Causal–pathogenic |
| c.437T>G | GTC-GGC | p.Val146Gly | EC | 9 | Familiar (2) | −5.32747 | Causal–pathogenic |
| c.645+1G>T | Donor splice site mutation | Exon 5 skipped or intron 5 retention | IC | 10 | *De novo* (1) | — | Causal–pathogenic |
| c.637G>C | GGG-CGG | p.Gly213Arg | IC | 11 | Familiar (3) | −2.8563 | Non-pathogenic |
| c.736G>A | GAT-AAT | p.Asp246Asn | IC | 12 | Familiar (2) | −3.0027 | Non-pathogenic |
| c.45 T>G | GCT-GCG | p.Ala15Ala | Signal peptide | 12 | Familiar (4) | — | Non-pathogenic |

Ig, immunoglobulin; EC, extracellular; IC, intracellular.
In family history, the number denotes relatives with the same mutation. The PANTHER scores are not possible to calculate for splice site or frame-shift mutations (only for missense mutations that cause the non-synonymous amino acid exchange).

found in patients with phenotype CMT1B, with age of clinical symptoms mostly in the second decade. The phenotype of these patients is mild and consistent within the affected families. The NCVs are decreased, but the motor nerve conduction velocity (MNCV) is still above 30 m/s. Families 8 and 9 were diagnosed as CMT patients only by chance during other medical examinations. Clinical findings are poor within these families, with only absent tendon reflexes. Electrophysiological examination clearly shows the demyelinating neuropathy (CMT1B) in all mutation carriers. Patients with CMT1B phenotype were familial except the splice mutation c.645+1G>T, where relatives were not available for testing.

The subPSEC values of the PANTHER program are below −3 and refer to the disease-causing mutations in five of the novel mutations [Table 1, (13)].

## Discussion

We provided clinical, genealogical and *in silico* evidence that 8 out of the 11 presented new *MPZ* mutations seem to be pathogenic and that 2 are non-pathogenic because they do not segregate with the CMT phenotype in the family. There is a tight genotype/phenotype correlation within the families as is typical in MPZ mutations.

Other pathogenic mutations were previously described at the same AA position for the five mutations we reported, namely: p.Leu48Pro – with a variable phenotype, p.Gly103Glu – DSN, p.Pro132Leu – CMT1B, p.Thr143Met – no clinical data published and p.Val146Phe – DSN (14–18). In codon 103, the phenotype is similar. In codons 132 and 146, it is different in our observation.

The 5′ splice site mutation c.645+1G>T was found in patient 10 with CMT1B phenotype. We assumed that intronic mutation can alter the final protein by the exon skipping of exon 5 with frame shift or the intron 5 retention. The c.584+2T>G of the 5′ splice site mutation in the *MPZ* gene was previously referred to by Sabet et al. (9). They proved the exon 4 skipping for this mutation by RNA analysis of dermal nerves. We cannot examine the patient's family because they refused further DNA testing.

Mutations p.Gly213Arg and p.Asp246Asn found in two families with axonal (family 11) and demyelinating (family 12) CMT seem to be non-pathogenic or at least not causal for CMT in these families, because these mutations do not segregate

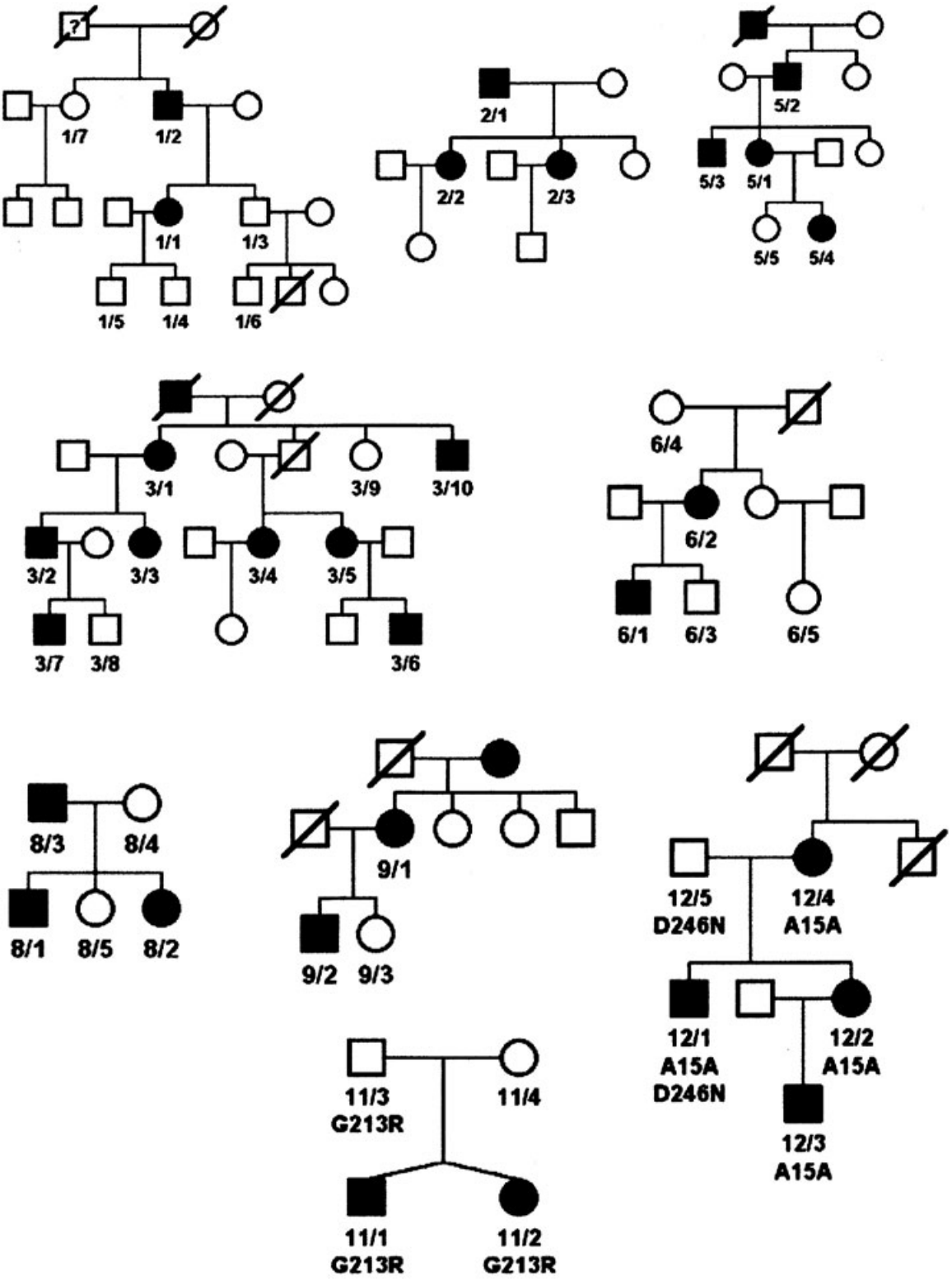


*Fig. 1.* Pedigrees of the reported families. Families 4, 7 and 10 are not shown as sporadic cases with *de novo* mutations. Square, man; circle, woman; black filled icon, affected patient; slash over the icon, deceased member. Individuals with numbers were examined in our laboratory. The first number is the number of the family and the number after the slash is the number of the patient. In families 11 and 12, where the mutations do not segregate with the phenotype, the genotypes for the available family members are shown.

Table 2. Clinical findings in patients with causal–pathogenic myelin protein zero (*MPZ*) mutations

| Family/patient | Gender | Age of onset (years) | Age of examination (years) | Feet deformities | LL reflexes | LL atrophy | UL atrophy | Walking ability | Phenotype | Age walked | Others |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1/1 | F | 49 | 51 | No | Absent | No | No | Normal | Sensory neuropathy | Normal | — |
| 2/1 | M | Second decade | 60 | Pes cavus | Absent | Severe | Severe | Cane support | CMT1B | Normal | — |
| 2/2 | F | n.d. | 35 | Mild pes cavus | Absent | No | No | Normal | CMT1B | Normal | — |
| 2/3 | F | n.d. | 38 | Pes cavus | Absent | Mild | No | Normal | CMT1B | Normal | — |
| 3/1 | F | Second decade | 54 | No | Absent | No | No | Normal | CMT1B | Normal | — |
| 3/2 | M | Second decade | 29 | Pes cavus | Absent | Mild | No | Normal | CMT1B | Normal | — |
| 3/3 | F | 24 | 28 | No | Absent | No | No | Normal | CMT1B | Normal | — |
| 3/4 | F | Asymptom. | 29 | Pes cavus | Absent | Mild | No | Normal | CMT1B | Normal | — |
| 3/5 | F | Second decade | 34 | Pes cavus | Absent | Mild | No | Normal | CMT1B | Normal | — |
| 3/6 | M | Asymptom. | 5 | Pes planovalgus | Absent | No | No | Normal | CMT1B | Delayed | — |
| 3/7 | M | Asymptom. | 3 | No | Normal | No | No | Normal | CMT1B | Normal | — |
| 4 | M | Infancy | 15 | Pes cavus | Absent | Mild | No | Instable walk | DSN/HMSN III | Delayed | Scoliosis |
| 5/1 | F | Second decade | 33 | High arched feet | Absent | No | No | Normal | CMT1B | Normal | — |
| 5/2 | M | Second decade | 68 | Pes equinus | Absent | Mild | Mild | Instable walk | CMT1B | Normal | — |
| 5/3 | M | Asymptom. | 43 | High arched feet | Decreased | No | No | Normal | CMT1B | Normal | — |
| 5/4 | F | Asymptom. | 9 | No | Decreased | No | No | Normal | CMT1B | Normal | — |
| 6/1 | M | Infancy | 20 | Pes cavus | Absent | Severe | No | Instable walk | DSN/HMSN III | Delayed | Scoliosis |
| 6/2 | F | Infancy | 51 | Pes cavus | Absent | Mild | No | Cane support | DSN/HMSN III | Delayed | Scoliosis |
| 7 | F | Infancy | 2 | Pes planovalgus | Absent | No | No | Instable walk | DSN/HMSN III | Delayed | Hypotonia |
| 8/1 | M | Asymptom. | 30 | No | Absent | No | No | Normal | CMT1B | Normal | — |
| 8/2 | F | Asymptom. | 37 | No | Absent | No | No | Normal | CMT1B | Normal | — |
| 8/3 | M | Asymptom. | 62 | No | Absent | No | No | Normal | CMT1B | Normal | — |
| 9/1 | F | 40 | 66 | High arched feet | Absent | No | No | Normal | CMT1B | Normal | — |
| 9/2 | M | Asymptom. | 34 | High arched feet | Absent | No | No | Normal | CMT1B | Normal | — |
| 10 | M | 12 | 22 | Pes cavus | n.d. | Mild | No | Normal | CMT1B | Normal | — |

CMT1B, Charcot-Marie-Tooth 1B; DSN, Dejerine-Sottas neuropathy; F, female; LL, lower limb; M, male; n.d., no data; UL, upper limb.

with the CMT disease. Mutation p.Gly213Arg was found in a clinically and electrophysiologically healthy father of age 50. Age of CMT2 onset in the children was about 6 years. CMT2 early onset was never reported as a phenotype of MPZ mutations. Mutation p.Asp246Asn was found also in the patient's healthy father and was not present in three other relatives also affected by CMT1 in the family. Our conclusion of non-pathogenic polymorphisms was supported by the score of the PANTHER program and the AA positions among species (Fig. S1). These variants were not found among 94 DNA controls and they are probably rare polymorphisms. Furthermore, a silent mutation p.Ala15Ala was found in all affected members of family 12, but it does not seem to create a new cryptic splice site. For final clarification, an RNA analysis from nerve tissue would be needed but is not available.

The PANTHER scores above −3 were also found for mutations in families 4 and 8. These values are in the range of non-pathogenic polymorphisms. However, we still think the mutations are causal and pathogenic because of the following facts. The p.Thr65Asn is a *de novo* mutation in a very conserved AA position among species and the patient suffers from DSS phenotype with extremely low NCVs in the upper limbs between 4–12 and 6–11 m/s and unrecordable NCVs in the lower limbs. The parents are healthy and do not carry the mutation. The AA position for the second p.Thr143Arg mutation, scored at the border of the probability for a polymorphism by PANTHER, is not so strongly conserved, but the mutation does segregate with the disease in this family. Furthermore, another mutation Thr143Met at the same codon was previously reported as pathogenic (18).

The question of whether or not the mutation p.Val46Met is pathogenic remains unanswered. The mutation was found in a patient with sensory loss at the age of 49 years and later with walking problems, but without muscle weakness. In contrast with late onset of the disease in MPZ-related CMT2, the electrophysiological results showed the demyelinating neuropathy with slight secondary axonopathy and only in the lower limbs. The patient's father is said to suffer from similar problems, with the age of onset around the fifth decade, but we did not have the opportunity to examine him. Another five members of this family are healthy, without this mutation. This phenotype is not typical for *MPZ* mutations; therefore, the cause for this sole sensory neuropathy in this patient is still to be determined. The Val46Met mutation could be present merely by chance as a rare non-pathogenic polymorphism. Final interpretation of this novel mutation would require another larger family, where segregation of the mutation with the phenotype could be followed or excluded.

Shy et al. (4) suggested that a large majority of patients with *MPZ* mutations have one of the two distinct phenotypes – early or late onset and only occasional patients have the 'classical CMT' phenotype. Among the mutations we reported, there is an unusually high frequency of CMT1B-associated phenotype. These patients have a minimal phenotype, with even subclinical findings as absent tendon reflexes, decreased NCVs and absent muscle weakness. The large number of CMT1B patients in our report could be due to DNA testing the patients with subclinical phenotype.

The reported genotype/phenotype correlations in larger families as we provided should help to provide proper genetic counselling in isolated patients worldwide in whom any of these mutations might be found.

## Supporting Information

The following Supporting information is available for this article:

Fig. S1. Sequence alignment of myelin protein zero (MPZ) proteins from different species. The position of the mutation is emphasized with a rectangle and the number with the amino acid position.

Table S1. Electrophysiological findings of available mutation carriers. NCS – nerve conduction standards – the standards of our laboratory for normal values

Additional Supporting information may be found in the online version of this article.

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## Conflict of interest

No competing financial interests exist for the main author and co-authors.

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