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CORRESPONDENCE

Expanding the phenotype and genotype spectra of *PLIN4*-associated myopathy with rimmed ubiquitin-positive autophagic vacuolation

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Recently, Ruggieri et al. [2] reported an autosomal dominant myopathy with rimmed ubiquitin-positive autophagic vacuolation (MRUPAV) with a coding 99-mer repeat-expansion in *PLIN4*, which was characterized clinically by weakness in distal muscles and pathologically by rimmed ubiquitin-positive autophagic vacuolation [2]. Here, we report that among myopathy patients at our neurology center, we found two families exhibiting marked proximal muscle weakness, ultimately finding that these families have repeat expansions in exon 3 of *PLIN4*. Notably, in one family, the number of repeat-expansions was higher than previous reports, and patients from this family had earlier onset and relatively more severe myopathy than previously reported cases.

Table 1 (Supplementary) presents detailed clinical characteristics of these patients. In Family 1, F1-III3 had proximal muscle weakness, predominantly in lower limbs, from age 58. Other affected family members reported slowly progressing proximal lower limb weakness beginning at age 45–65. Serum creatine kinase (CK) levels were normal or mildly elevated; electromyography (EMG) showed myopathic changes and myotonic discharges; some patients (F1-III4, F1-III5, and F1-III6) were asymptomatic but showed myopathic changes in their EMG. In Family 2, F2-III4 developed proximal muscle weakness in lower limbs from age 25, followed by muscle involvement in the proximal upper limb, and the distal muscles were also partially involved. He lost his mobility at age 39 and relied on a wheelchair. Other affected individuals in his family reported muscle weakness beginning in their twenties. The CK levels of F2-III4 and F2-III6 were mildly elevated and EMG showed myopathic changes. None of our patients had bulbar symptoms or respiratory abnormalities. Patients in both families were mainly characterized by progressive proximal weakness; this is distinct from previous reports of MRUPAV patients, which were mainly characterized by progressive distal weakness [1, 2]. Therefore, both the onset symptoms and pattern of muscle involvement in our two families were distinct from the MRUPAV patients reported previously. Patients in Family 2 had earlier age at onset (25–26 in Family 2 vs 45–65 in Family 1), more severe proximal limb weakness, and faster disease progression than patients in Family 1 and the previous reported cases.

In Family 1, muscle imaging showed mild to severe fatty replacement, and calf muscles were more involved than thigh muscles. In F2-III6, there was only mild fatty infiltration of the thighs and calves at 3 years after onset (at 26 years). Histopathology including haematoxylin and eosin and modified Gomori trichrome staining showed myopathic changes to various degrees, characterized by fiber size variation, endomysial fibrosis, and rimmed vacuoles (Fig. 1g–n), signs similar to the previously reported cases. Oil Red O and periodic acid–Schiff staining were normal (Fig. 1o and p). Some fibers showed small focal areas devoid of NADH-tetrazolium reductase activity (Fig. 1q). Cytochrome c oxidase/succinate dehydrogenase (COX/SDH) double-staining occasionally showed COX deficient fibers which appeared blue (Fig. 1r). Electron microscopy revealed vacuoles filled with membranous bodies, partially degraded organelles, and amorphous or granular material, which often abutted the extracellular space (Supplementary Fig. 1).

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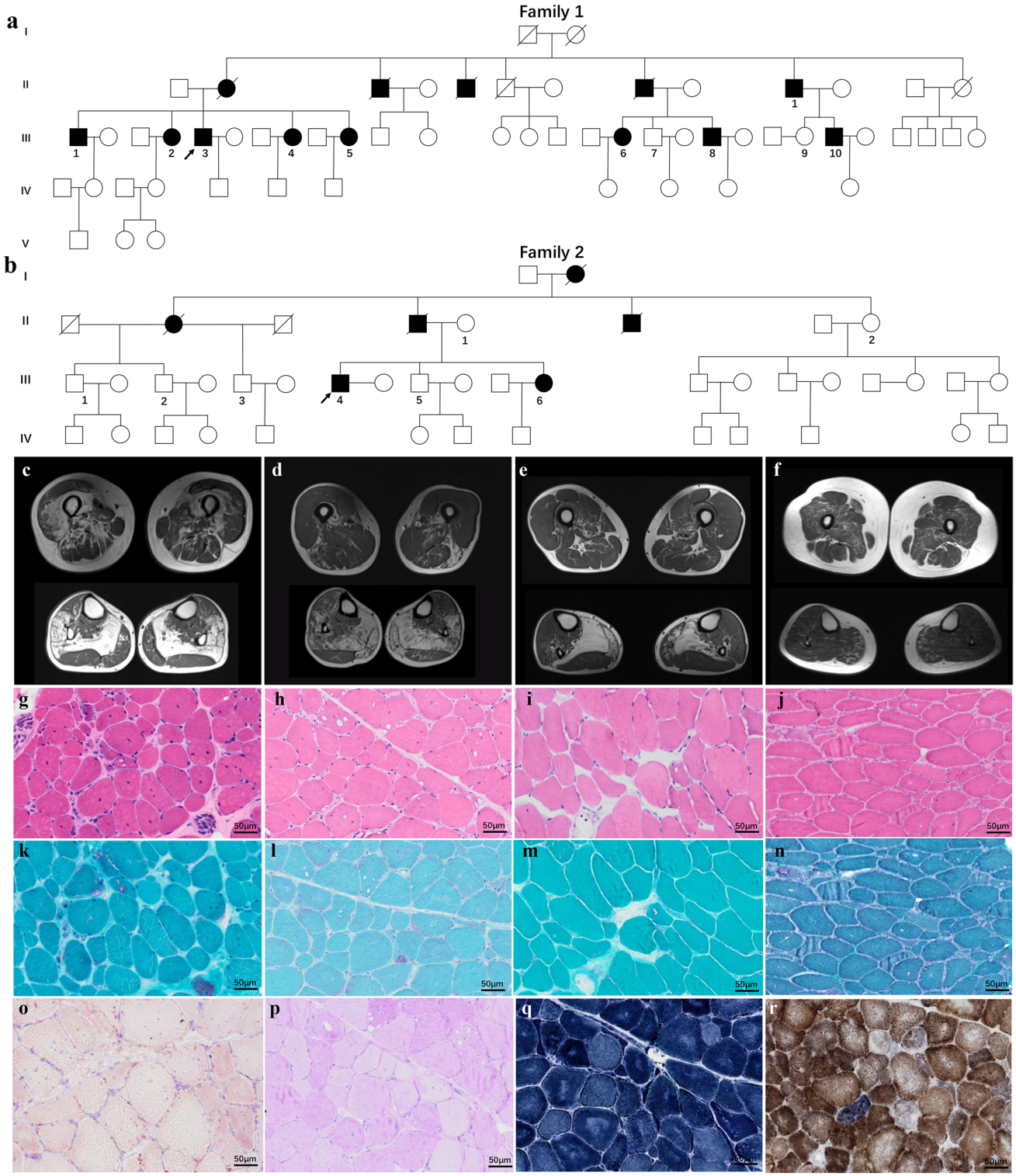

Fig. 1 Pedigrees, patient muscle imaging, and pathology. **a** and **b** Pedigree chart of the two *PLIN4*-related MRUPAV families. **c**–**f** Thigh and calf muscle MRI (T1-weighted images) of F1-III1 (**c**), F1-III3 (**d**), F1-III8 (**e**), and F2-III6 (**f**). **g**–**n** Haematoxylin and eosin and modified Gomori trichrome staining of muscles biopsies from F1-III1 (**g** and **k**), F1-III3 (**h** and **l**), F1-III8 (**i** and **m**), and F2-III4 (**j** and **n**). **o**–**q** Histochemistry images of muscle from F1-III3. Oil Red O (**o**), Periodic acid–Schiff (**p**), NADH-tetrazolium reductase (**q**), cytochrome c oxidase/succinate dehydrogenase (COX/SDH) double-labeling (**r**)

All known genes associated with neuromuscular disorders were negative based on screening with whole-exome and whole-genome sequencing. We subsequently sampled additional members of Family 1 and performed parametric linkage analysis using Merlin (v1.1.2), which identified a 0.3 MB candidate region at 19p13.3-19p13.2 (chr19: 4,186,395–7,166,541) with a maximum logarithm of odds score of 3.3082 (Supplementary Fig. 2). Next, we conducted Oxford Nanopore long-read whole-genome sequencing of F1-III3 and F2-III4. In the candidate region, we found both patients harbored multiple reads with abnormal 99-mer repeat expansions in exon3 of *PLIN4*, which was absent from the healthy individual; F2-III4's 99-mer repeat size was larger than F1-III3's repeat (Supplementary Fig. 3). We amplified the *PLIN4* repeat region of exon 3: unaffected individuals had a single band (~3000 bp), while affected individuals in Family 1 had this wild-type 3000 bp band and a second ~4000 bp band; this second band is similar to previously reported *PLIN4*-related MRUPAV cases. Affected individuals in Family 2 had a wild-type band and a second, ~5000 bp band (Supplementary Fig. 4). Ultimately, patients from these two families were diagnosed with *PLIN4*-related MRUPAV.

It seems plausible that the higher number of repeat expansions in Family 2 may explain their earlier age at onset, more severe phenotypes, and faster disease progression. Consistent with previous reports, an immunofluorescence analysis revealed FK2 and p62/SQSTM1 positivity in our patients' vacuoles and subsarcolemmae. Moreover, in patient muscle perilipin-4 co-localized with FK2 and p62/SQSTM1, as well as with the aggrephagy-related proteins NBR1 and LC3B (Supplementary Fig. 5), suggesting that repeat expansion is associated with increased perilipin-4 aggregation and aggrephagy.

In summary, we studied two families with myopathy related to a coding 99-mer repeat-expansion in *PLIN4*, thus corroborating previous reports. It was interesting to note that the size of the expansion was strongly correlated to the age at onset and the severity of the disorder, and we observed proximal rather than distal myopathy for *PLIN4*-related MRUPAV.

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Declarations

Conflict of interest The authors disclose no conflicts of interest.

References

1. Di Blasi C, Moghadaszadeh B, Ciano C, Negri T, Giavazzi A, Cornelio F et al (2004) Abnormal lysosomal and ubiquitin-proteasome pathways in 19p13.3 distal myopathy. Ann Neurol 56:133–138. https://doi.org/10.1002/ana.20158
2. Ruggieri A, Naumenko S, Smith MA, Iannibelli E, Blasevich F, Bragato C et al (2020) Multiomic elucidation of a coding 99-mer repeat-expansion skeletal muscle disease. Acta Neuropathol 140:231–235. https://doi.org/10.1007/s00401-020-02164-4