Genotype–phenotype characteristics of Chinese Charcot–Marie–Tooth disease type 2A and related MRI features

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To the Editor: Charcot–Marie–Tooth disease type 2A (CMT2A), caused by mutations in Mitofusin-2 (*MFN2*), is the most common form of axonal CMT, accounting for 20–30% of CMT2.[1,2] CMT2A exhibits clinical and genetic heterogeneity, with most disease-causing mutations located within the conserved guanosine triphosphatase (GTPase) domains.[3] Intra-familial variability within the family has been observed. Consequently, comprehensive genotype–phenotype studies of CMT2A, particularly in large cohorts, are essential for advancing the understanding of CMT. Additionally, qualitative muscle magnetic resonance imaging (MRI) has been used to assess disease severity in CMT.[4] However, studies on intramuscular fat fraction (FF), a potential sensitive marker, in CMT2A patients remain scarce in the literature.

In this study, we described the clinical and genetic features of 113 patients with *MFN2* mutations and analyzed available muscle MRI data. We aimed to characterize the genotype–phenotype correlations in CMT2A and explore muscle FF as a potential biomarker for CMT clinical trials.

A total of 269 CMT2 families from Central South China were enrolled from the Third Xiangya Hospital between 2012 and 2023. All index patients and available family members underwent complete neurological examinations. Disease severity was assessed using the CMT neuropathy score and the CMT examination score (CMTES). This study was approved by the Institutional Review Board of the Third Xiangya Hospital of Central South University (No. 21021). Written informed consent was obtained from all participants. All CMT2 patients underwent direct *MFN2* Sanger sequencing or next-generation sequencing. Thirteen patients underwent MRI scans of calf and thigh muscles on MRI 3T. Clinical evaluations, genetic analyses, MRI protocols, and statistical analysis are listed in Supplementary Methods, http://links.lww.com/CM9/C420.

Of 269 CMT2 families, 66 (24.5%) confirmed harboring *MFN2* variants. Forty distinct pathogenic or likely pathogenic variants in *MFN2* were identified, including three novel variants: c.649T>G (p.C217G), c.657T>G (p.D219E), and c.2204dupG (p.R735fs) [Figure 1A, Supplementary Table 1, http://links.lww.com/CM9/C420]. Notably, *de novo* mutations were identified in 27 CMT2A patients (40.9%, 27/66). The p.R94W/Q variant was detected in 16 unrelated families (24.2%, 16/66).

A total of 113 patients from the 66 CMT2A families were recruited. Ninety-two patients (81.4%) experienced disease onset in the first decade and exhibited mild to moderate severity, while 21 patients (18.6%) developed symptoms after age of 10 years, showing a milder phenotype [Supplementary Table 2, http://links.lww.com/CM9/C420]. The early-onset group had a significantly higher CMTES than the late-onset group ($P = 0.014$; Supplementary Figure 1A, http://links.lww.com/CM9/C420). However, disease progression was similar across both groups [Figure 1B].

Family history indicated autosomal dominant (AD) inheritance in 43.9% (29/66) and simplex cases in 56.1% (37/66) of families. Sporadic CMT2A patients showed earlier disease onset compared to AD patients ($P < 0.001$), with no significant differences in baseline CMTES between two groups [Supplementary Figure 1B, C, http://links.lww.com/CM9/C420]. To minimize potential

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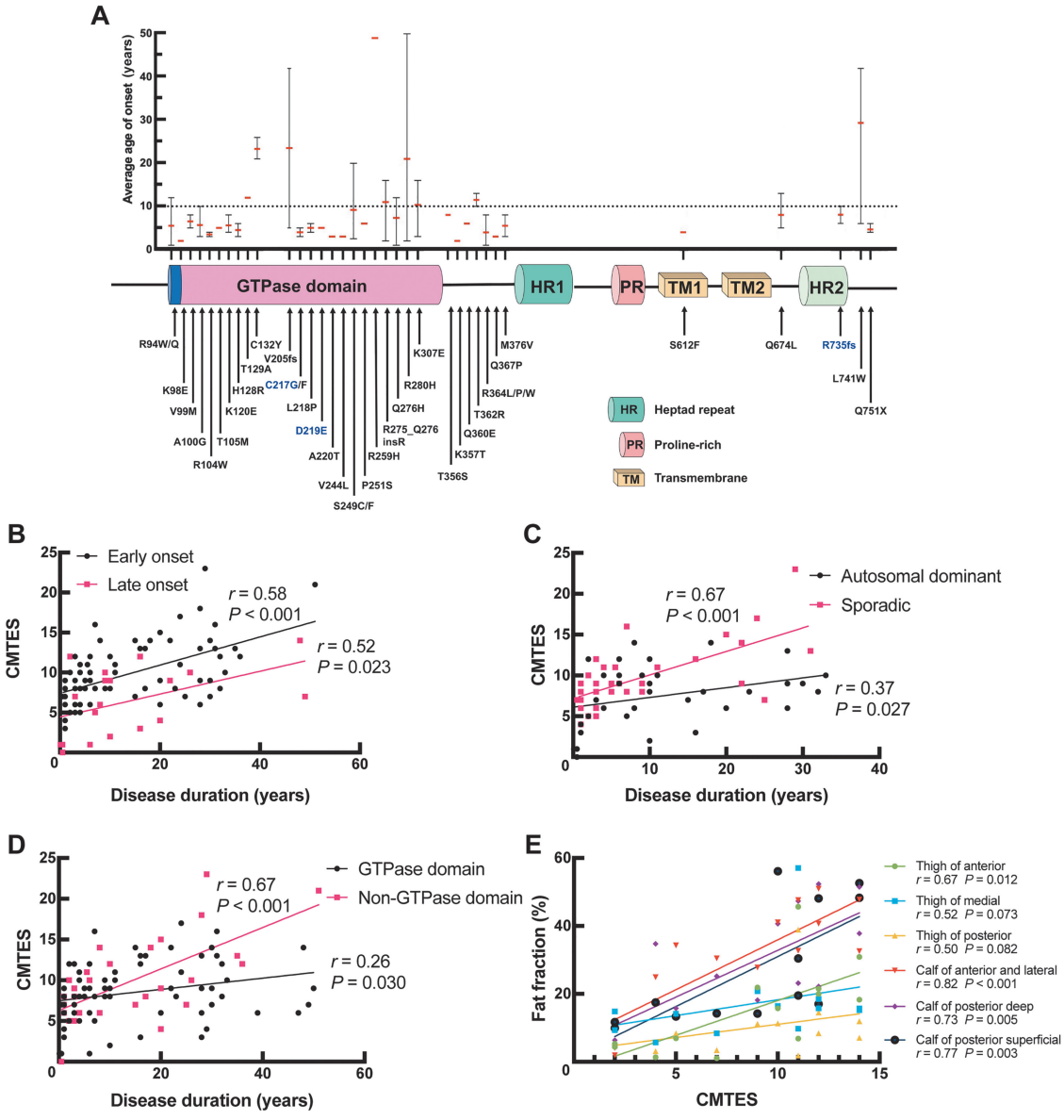

Figure 1: (A) Schematic of 40 *MFN2* variants identified in our cohort and the average age of onset at each variant. The MFN2 protein structure below highlights the functional domains where the variants are located. Blue indicates novel variants detected in this study. (B–D) Correlations of CMTES and disease duration between the different subgroups. (E) Correlations between muscle fat fraction and CMTES for muscle compartment. CMTES: Charcot-Marie-Tooth examination score; GTPase: Guanosine triphosphatase; MFN2: Mitofusin-2.

confounding and selection bias, propensity score matching was performed, resulting in matched sporadic and AD patients with comparable disease durations (1:1 ratio; 4.0 [1.0–11.0] years *vs.* 10.0 [4.0–20.0] years). Regression analysis showed that sporadic cases ($r = 0.67$, $P < 0.001$) progressed faster than AD patients ($r = 0.37$, $P = 0.027$; Figure 1C). Similar trends were observed between *de novo* and AD groups [Supplementary Figure 1D, E, http://links.lww.com/CM9/C420].

The correlation between the age at onset (AAO) or severity and each variant was also explored. Most variants were associated with early onset, whereas patients with p.T129A, p.C132Y, and p.R259H mutations had later onset [Figure 1A]. Hotspot mutations p.R94W/Q were correlated with early onset, however, the disease severity varied, ranging from mild to severe (CMTES 1–15; Supplementary Table 3, http://links.lww.com/CM9/C420). Patients with non-GTPase domain variants showed accelerated disease progression ($r = 0.67$, $P < 0.001$) compared to GTPase domain variants ($r = 0.26$, $P = 0.030$; Figure 1D), though no differences in AAO or baseline CMTES were observed between groups [Supplementary Figure 1F, G, http://links.lww.com/CM9/C420].

Marked phenotype variability in CMT2A was observed. For example, p.V205fs, p.S249C, p.R280H, and p.L741W were associated with both early onset, mild severity and late-onset, moderate disease. We observed significant intra-familial variability in disease onset and progression in the family carrying p.S249C and p.L741W variants. Additionally, two probands with heterozygous *MFN2*/Ganglioside-induced differentiation associated Protein-1 (*GDAP1*) concomitant variants showed earlier onset and

more severe phenotypes than their parents with a single *MFN2* variant.

Thirteen CMT2A patients underwent muscle MRI scans [Supplementary Figure 2A, http://links.lww.com/CM9/C420], revealing significantly higher FF values in the calf (29.42 ± 15.02%) than the thigh (13.72 ± 11.12%). Control subjects exhibited low FF values in both the thigh (4.17 ± 1.74%) and calf (2.72 ± 0.84%). The anterior/lateral calf compartments showed the greatest degree of involvement in patients [Supplementary Figure 2B, C, http://links.lww.com/CM9/C420]. The strongest correlation between CMTES and FF was observed in the anterior/lateral calf compartment ($r = 0.82$; $P < 0.001$; Figure 1E), with tibialis anterior ($r = 0.86$; $P = 0.001$) and peroneus longus ($r = 0.84$; $P < 0.001$) showing the highest correlations among individual muscles [Supplementary Figure 2D, http://links.lww.com/CM9/C420].

This study demonstrated a comprehensive clinical profile of 113 CMT2A patients from Central South China, which is a large-scale *MFN2*-related cohort study in Chinese mainland. The frequency of *MFN2* mutations was 24.5% of CMT2, which is higher than that in North China but similar to findings in other geographical populations.[1,5,6] We reported three novel *MFN2* variants, expanding the *MFN2*-related genetic spectrum.

There was significant phenotypic heterogeneity with respect to both the AAO and disease severity. The p.R94Q/W hotspot mutation was associated with early onset, mild to severe neuropathy. Intra-familial variability was marked in cases with the p.V205fs, p.S249C, p.R280H, p.K307E, and p.L741W variants. Additionally, concomitant *MFN2*/*GDAP1* variants were identified in our cohort. These results highlight the clinical heterogeneity of CMT2A.

Patients with non-GTPase domain variants developed more rapid disease progression, as the non-GTPase domain is involved in maintaining the structural stability of the MFN2 protein and interacting with other proteins. Mutations in the non-GTPase domain may accelerate disease progression by disrupting mitochondrial dynamics and protein stability. The proportion of sporadic cases exceeded than that of family cases, with sporadic patients demonstrating faster disease progression. *De novo* mutations are commonly reported in CMT. In our cohort, 40.9% of CMT2A patients harbored *de novo* mutations, consistent with a North China study.[6] These complex issues for affected families complicate genetic counseling.

Quantitative MRI of muscle FF revealed severe involvement in the calf muscles and mildly affected in the thigh muscles in CMT2A patients, aligning with the pattern of motor deficits extending from distal to proximal. The MRI findings reflected a distinct pattern of muscle involvement, with the anterior/lateral calf compartments showing the greatest impact, where FF changes strongly correlated with disease severity. Taken together, quantitative MRI analysis of lower limb muscle FF could serve as a sensitive biomarker targeting CMT2A.

In conclusion, we reported novel pathogenic *MFN2* mutations and described the clinical characteristics in terms of age of onset, inheritance patterns, and variant topologies, expanding the genetic and clinical spectrum of *MFN2*. Our study revealed that calf compartment-specific FF could be a biomarker, with potential applications for assessing CMT2A patients in clinical trials.

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Conflicts of interest

None.

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