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CORRESPONDENCE

CASQ1 mutations impair calsequestrin polymerization and cause tubular aggregate myopathy

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Tubular aggregate myopathy (TAM) is a rare muscle disorder characterized by abnormal accumulations of membrane tubules in muscle fibers, and marked by progressive muscle weakness, cramps, and myalgia [3]. Genetically, TAM has been assigned to mutations in *STIM1* [2] and *ORAI1* [7], both encoding key regulators of Ca^{2+} homeostasis. Through exome sequencing of molecularly undiagnosed TAM cases, we now identified *CASQ1* as the third TAM gene, and we support our findings by clinical, histological, genetic, and functional data.

Family 1 has an ancestral history of a muscle phenotype segregating as a dominant disease, and a partial clinical and histological description was reported earlier [8]. Patient 103901 from Family 2 is a singleton. Birth, early childhood, and motor milestones were normal for all affected members from both families. Disease onset was between early 20s and mid-40s with a slowly progressive muscle weakness mainly involving the proximal muscles in the lower limbs for Family 1, and early 50s with post-exercise myalgia in the lower limbs for Family 2 (Supplementary Table 1). Histological and ultrastructural analyses of the muscle biopsies displayed tubular aggregates as the main histopathological hallmark in both families (Fig. 1a). Exome sequencing identified the heterozygous *CASQ1* missense mutations c.166A>T (N56Y) in exon 1 in Family 1, and c.308G>A (G103D) in exon 2 in Family 2. Both mutations affect highly conserved amino acids (Supp. Figure 1), none was found in the available healthy family members, and none was listed in the public or internal SNP databases. A single *CASQ1* missense mutation (D244G) has previously been associated with vacuolar myopathy involving protein aggregates [9]. *CASQ1* is primarily expressed in skeletal muscle and encodes calsequestrin, the major Ca^{2+} storage protein in the sarcoplasmic reticulum. Calsequestrin binds Ca^{2+} with moderate affinity and high capacity, and forms higher order polymers with increasing Ca^{2+}-binding capacities [4].

Immunohistochemistry on a muscle biopsy from Family 2 revealed strong signals for calsequestrin, STIM1, and RyR1 in aggregated structures most likely corresponding to the tubular aggregates, while ORAI1 was not trapped (Fig. 1b). This conforms to the observations made on biopsies from *STIM1* and *ORAI1* patients and demonstrates that the trapped proteins are primarily of sarcoplasmic reticulum origin [1, 2]. These findings on a single muscle biopsy also suggest that aggregation of STIM1 appears to be a consequence of *CASQ1* mutations, providing a pathological link between *STIM1*- and *CASQ1*-related TAM. In transfected C2C12 myoblasts, WT and both TAM N56Y and G103D mutants formed calsequestrin networks of comparable complexity, while the vacuolar myopathy D244G mutant induced major calsequestrin aggregation (Fig. 1c). Calsequestrin polymerization and depolymerization are dynamic

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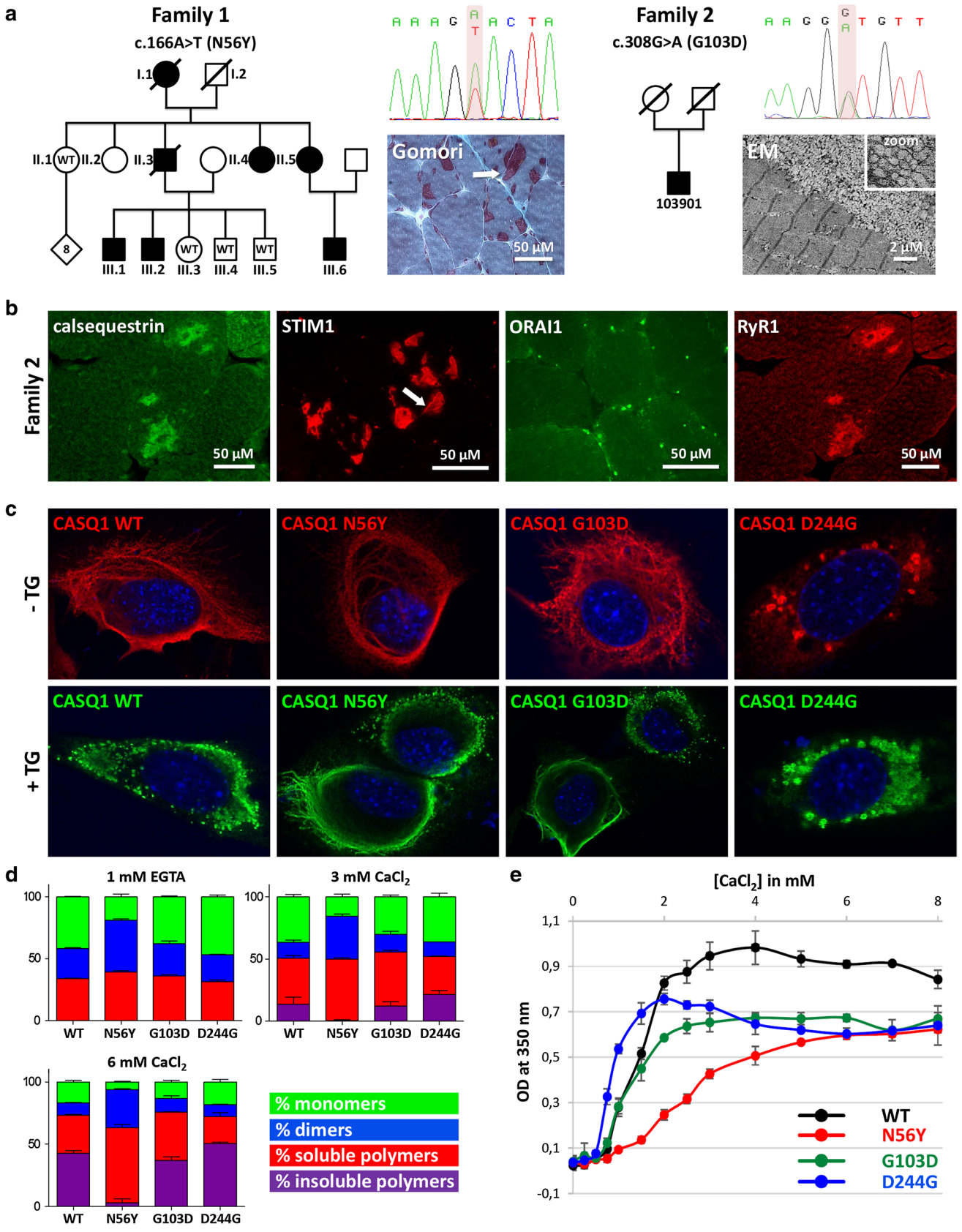
a
Family 1
c.166A>T (N56Y)
I.1
I.2
II.1
WT
II.2
II.3
II.4
II.5
8
III.1
III.2
III.3
III.4
III.5
III.6
Gomori
50 µM
Family 2
c.308G>A (G103D)
103901
EM
zoom
2 µM
b
Family 2
calsequestrin
STIM1
ORAI1
RyR1
50 µM
c
- TG
+ TG
CASQ1 WT
CASQ1 N56Y
CASQ1 G103D
CASQ1 D244G
d
1 mM EGTA
3 mM CaCl2
6 mM CaCl2
100
50
0
WT
N56Y
G103D
D244G
% monomers
% dimers
% soluble polymers
% insoluble polymers
e
[CaCl2] in mM
0
2
4
6
8
OD at 350 nm
1,1
0,9
0,7
0,5
0,3
0,1
-0,1
WT
N56Y
G103D
D244G

◂**Fig. 1** Impact of the *CASQ1* mutations and immunohistochemistry on muscle biopsies. **a** Heterozygous *CASQ1* missense mutations segregating with the disease were identified in both families displaying tubular aggregates on muscle biopsies (arrow, zoom). **b** Immunohistochemistry on the biopsy from Family 2 revealed aggregation of calsequestrin, STIM1, and RyR1, but not of ORAI1. The STIM1 signals were thereby more pronounced in the periphery of the aggregated structures (arrow), while calsequestrin and RyR1 were also found in the center. **c** In Ca^{2+}-containing medium, WT calsequestrin and the N56Y and G103D mutants formed complex networks in transfected C2C12 myoblasts, while the D244G mutant aggregated. Ca^{2+} store depletion through addition of Thapsigargin (Tg) induced major monomerization of WT calsequestrin, while the N56Y and G103D mutants remained in large parts polymeric. **d** Analytical ultracentrifugation showed that especially the TAM N56Y mutant formed less insoluble higher order polymers at rising Ca^{2+} concentrations compared to the wild type, while the vacuolar myopathy D244G strongly polymerized. **e** Turbidity assays confirmed the reduced propensity of both TAM mutants to polymerize at rising Ca^{2+} concentrations

Ca^{2+}-dependent processes, and the depletion of the SR Ca^{2+} stores was shown to favor the monomeric form of calsequestrin [6]. To test whether the *CASQ1* mutations influence calsequestrin monomerization upon Ca^{2+} store depletion, we treated the transfected cells ($n > 300$ per condition) with the SERCA inhibitor Thapsigargin (Tg). Dot-like calsequestrin signals corresponding to monomers or minor oligomers were seen in the majority (76%) of the cells expressing the WT, but only in 49.4% and 42.5% of the cells, respectively, expressing N56Y or G103D. To investigate the impact of the *CASQ1* mutations on calsequestrin polymerization, we performed analytical ultracentrifugation in solution containing recombinant calsequestrin and rising Ca^{2+} concentrations (Fig. 1d). We found that especially the TAM N56Y mutant formed significantly less higher order polymers than the wild-type, while the vacuolar myopathy D244G mutant showed an increased propensity to form insoluble polymers. To follow the polymerization kinetics of WT and mutant calsequestrin, we next performed turbidity assays (Fig. 1e). G103D and especially N56Y calsequestrin show reduced polymerization rates, and produced significantly less polymers at maximal Ca^{2+} levels compared to the wild-type protein. Contrasting wild-type and TAM calsequestrin, the D244G mutant strongly polymerized at minimal Ca^{2+} levels, confirming the results obtained by a previous study [5].

Taken together, we demonstrate that specific *CASQ1* mutations are one of the genetic causes of TAM. We show that the tubular aggregates in our patients contain calsequestrin, STIM1, and RyR1, and we provide functional evidence that *CASQ1* mutations significantly impair calsequestrin polymerization and depolymerization, while the vacuolar myopathy mutation increases Ca^{2+}-dependent polymerization. This suggests that the *CASQ1* mutations causing either TAM or vacuolar myopathy involve different pathomechanisms.

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