Supporting Information

Page et al. 10.1073/pnas.0811753106

SI Methods

Generation of Transgenic D187N Plasma Gelsolin Mice. D187N plasma gelsolin cDNA was amplified by PCR to contain a Kozak sequence at the 5′ end and was inserted into a vector containing the muscle creatine kinase (MCK) promoter, −3300 to +7 (a gift from S. Hauschka, University of Washington, Seattle, WA) (1). A rabbit globin intron inserted just 5′ to the plasma gelsolin gene and a bovine growth hormone polyadenylation signal at the 3′ end was kindly provided by J. Conkright (Scripps Florida, Jupiter, FL) (2). The transgene was sequenced to verify the fidelity of the PCR. To generate transgenic mice, the transgene was excised from the vector and purified. Pronuclear injections into the C57BL/6J strain were performed at the mouse genetics core facility at The Scripps Research Institute. Founder mice were identified by transgene-specific PCR. Two of 4 founder mice both transmitted and expressed the human D187N plasma gelsolin transgene. All data were derived from mice that were from generation F_4 or above. The data shown herein are from mice derived from only one of these original founders, founder A. The female and male mice from this strain had similar D187N gelsolin levels, consistent with insertion into one of the autosomic chromosomes. However, mice derived from founder B showed sex-linked transmission of the transgene indicating insertion in the X chromosome. In this strain, the male mice had similar D187N gelsolin expression levels as in strain A; however, female mice had approximately one-half the levels because of the random switching off of the X chromosome. Both strains exhibited gelsolin amyloidosis in the endomysium of muscle fibers, and gradually, as the mice aged, developed intracellular inclusions, indicating that the location of transgene insertion was not affecting the observed phenotypes and pathology. However, we did observe that female mice from strain B were delayed in the development of this phenotype, consistent with the lower levels of D187N gelsolin expressed.

The hemizygous D187N (−/+) mice (strain A) were crossed with each other to obtain homozygous D187N (+/+) animals. Backcrossing to wild-type C57BL/6J was undertaken to confirm the homozygous (+/+) state.

Immunoblots. Mice were saline perfused and isolated tissues homogenized in PBS containing protease inhibitors (Sigma) by using a Teflon mechanical homogenizer at 70 rpm. Protein concentrations were determined using Pierce BCA (Pierce) and equal amounts loaded onto SDS/PAGE gels (Tris-tricine gels were used for the small peptides as described in ref. 3). An anti-FAF antibody [directed against the human 8-kDa amyloidogenic peptide (4) and does not recognize mouse gelsolin] or anti-APP (CT695; Invitrogen) was used. For detection of Aβ in muscle, the homogenates were centrifuged at 20,000*g* for 10 min and the pellet was resuspended in formic acid for at least 1 h at room temperature. After lyophilization, the sample was resuspended in 0.1 M NaOH, 0.1% SDS for 5 min before the addition of sample buffer. After the transfer of the proteins from gels to blots, the blots were boiled for 10 min in PBS before being exposed to antibody 4G8 (Sigma).

Light and Electron Microscopy. Muscle specimens (vastus lateralis, cranial tibial) were frozen in isopentane precooled in liquid nitrogen immediately following humane euthanasia. Unfixed cryosections (8 μm) were stained with H&E for general morphology and for Congo red fluorescence localization using rhodamine optics. For ultrastructural analysis by electron microscopy, glutaraldehyde-fixed muscle specimens were postfixed in 1% aqueous osmium tetroxide before dehydration and embedding in Araldite resin. Thick sections (1 μm) were stained with toluidine blue-basic fuchsin before light microscopic examination, whereas thin sections (60–90 nm) were stained with uranyl acetate and lead citrate before examination in a Zeiss 10 electron microscope.

Immunofluorescence. Unfixed cryosections (8 μm) were stained with antibodies against anti-FAF (4), anti-APP (Invitrogen), anti-Aβ 1–42 (Abcam), anti-ubiquitin (Dako), and anti-α-sarcoglycan (a gift from E. Engvall, The Burnham Institute for Medical Research, La Jolla, CA).

Amyloid Isolation from Skeletal Muscle Tissues. Skeletal muscle tissue specimens, obtained from 18-month-old wild-type and D187N (−/+) transgenic mice, were frozen at −70 °C until further processed. Using a Teflon mechanical homogenizer (80 rpm) at 4 °C, thawed muscle tissues were minced and homogenized in 150 mM NaCl until no solid tissue was visible. Amyloid was isolated from the tissues as described in ref. 5.

Immunogold Electron Microscopy. Parlodion-coated nickel grids were glow discharged and inverted onto droplets of amyloid suspension. After a brief wash in 0.05 M Tris buffer, the samples were blocked in 10% FCS in Tris, incubated for 2 h in anti-FAF in 5% FCS at room temperature, and washed multiple times in 2% FCS. Subsequent treatment consisted of incubating the grids in protein A-gold (10 nm) (J. Slot, Department of Cell Biology, University of Utrecht, Utrecht, The Netherlands) diluted in 2% FCS in Tris buffer. The grids were then washed multiple times in 0.05 M Tris·HCl, pH 7.4, plus 0.05% Tween 20, fixed in 1% glutaraldehyde in PBS, and washed twice in water before negative staining in 1% aqueous uranyl acetate and viewing with a Philips CM100 electron microscope (FEI).

Grip Strength. To assess grip strength, each mouse was held by the base of the tail and the forelimbs placed on the bar of a grip strength meter so that the forelimbs supported the weight of the mouse. Baseline force in grams was recorded and then the mouse was gently pulled by the base of the tail until the animal's grip was released from the bar. The force at which the animal released its grip was measured for 5 trials and then averaged (6). Group means were analyzed with ANOVA followed by Fisher's least significant difference (LSD).

Challenging Beam Traversal. Motor performance was measured with a challenging beam traversal test as previously described in refs. 7 and 8. Briefly, the beam was constructed out of Plexiglas (Plastics Zone) and consisted of four sections (25 cm each, 1 m total length), each section having a different width. The beam started at a width of 3.5 cm and gradually narrowed to 0.5 cm by 1 cm decrements. Support ledges were attached along each side of the beam. Animals were trained to traverse the length of the beam starting at the widest section and ending at the narrowest, most difficult, section. The narrow end of the beam led directly into the animal's home cage. Animals received 2 days of training before testing, and all training was performed without the mesh grid. On the test day, a mesh grid (1-cm squares) of corresponding width was placed over the beam surface leaving approximately a 1-cm space between the grid and the beam surface. Animals were then videotaped while traversing the grid-surfaced

beam for a total of 5 trials. All animals were tested on the beam with mesh grid.

Videotapes were viewed and rated in slow motion for errors, number of steps made by each animal, and time to traverse across by an investigator blind to the mouse genotype. Error per step scores, time to traverse, and number of steps were calculated for all mice across all 5 trials and averaged. Group means were analyzed by using ANOVA followed by Fisher's LSD.

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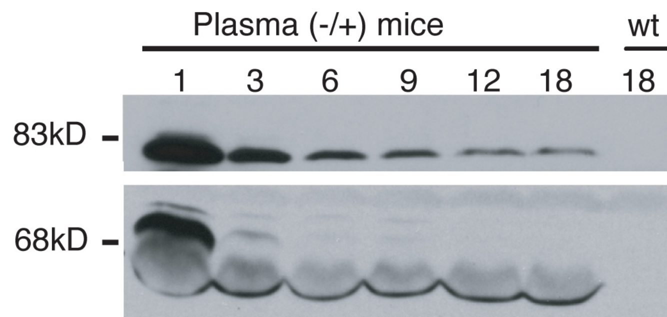

Fig. S1. Plasma levels of human D187N gelsolin decrease with age. Longer exposure of Fig. 1*E* (*Upper*) and a shorter exposure of Fig. 1*E* (*Lower*) indicate that the plasma levels of the full-length 83- and 68-kDa fragment behave in a similar manner.

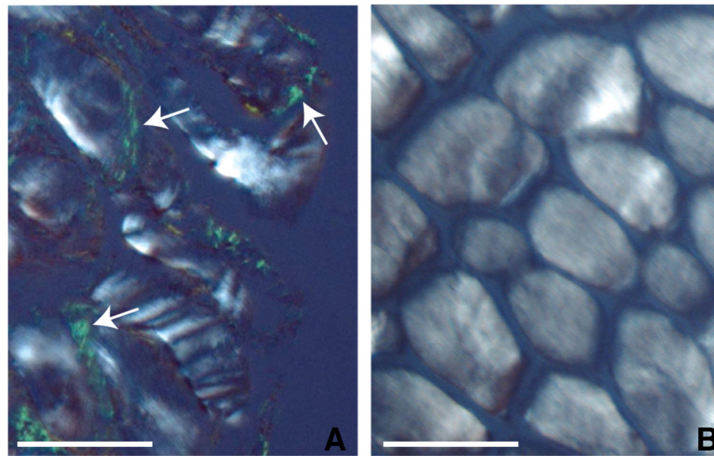

Fig. S2. Amyloid detected in D187N muscle. (*A* and *B*) Skeletal muscle, fixed, embedded, and sectioned was incubated with Congo red and analyzed by light microscopy using polarized light. Apple-green birefringence generated by amyloid was observed in muscle from D187N (−/+) mice (*A*, indicated with arrows) but not wild-type mice (*B*). (Scale bars: 50 μm.)

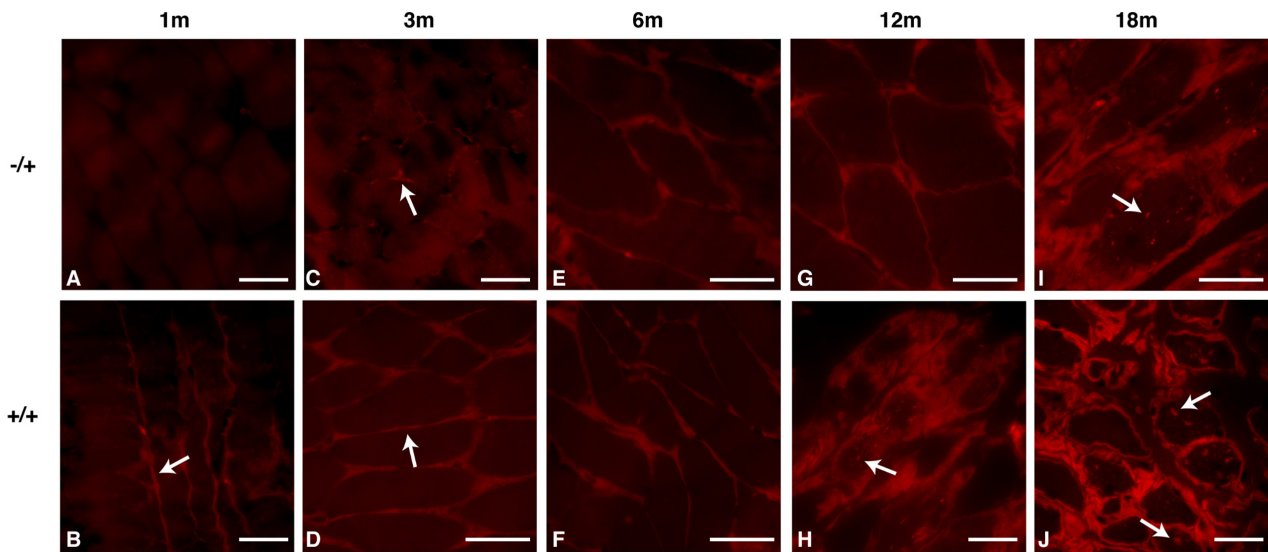

Fig. S3. Age-dependent amyloid deposition in hemizygous (−/+) and homozygous (+/+) D187N gelsolin mice. Muscle specimens obtained from both (−/+) and (+/+) D187N mice were evaluated for CR fluorescence at 1 (*A* and *B*), 3 (*C* and *D*), 6 (*E* and *F*), 12 (*G* and *H*), and 18 (*I* and *J*) months of age. Although no deposits are detected in 1-month-old (−/+) mice (*A*), some small areas of Congo red fluorescence, indicative of amyloid, were detected in 1-month-old D187N (+/+) mice (*B*, arrow). By 3 months of age, D187N (−/+) mice showed deposition of amyloid around capillaries (*C*, arrow), whereas the deposition in 3-month-old D187N (+/+) mice was much more widespread and extended into the endomysium (*D*, arrow). By 9–12 months of age, D187N (+/+) mice revealed the presence of intracellular inclusions (*H*, arrow) that were not detected in D187N (−/+) mice of the same age (*G*). Intracellular inclusions were detected in D187N (−/+) animals at 18 months of age (*I*, arrow), but were substantially more extensive in 18-month-old D187N (+/+) mice (*J*, arrow). (Scale bars: 50 μm.)

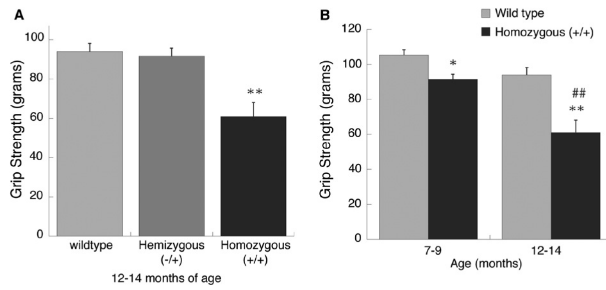

Fig. S4. Homozygous D187N gelsolin (+/+) mice exhibit muscle weakness. (*A*) Grip strength was compared between wild type (*n* = 21; females, 13; males, 8), hemizygous D187N (−/+; *n* = 20; females, 10; males, 10), and homozygous D187N (+/+; *n* = 10; females, 5; males, 5) mice at 12–14 months of age. No significant difference between wild-type and hemizygous (−/+) mice was detected, whereas the grip strength of 12- to 14-month-old homozygous (+/+) mice was significantly reduced compared to both wild-type and hemizygous mice (*A* and *B*; **, $P < 0.01$; $F_{(2,50)} = 11.27$). (*B*) Grip strength was also significantly reduced in homozygous D187N (+/+) mice (*n* = 12; females, 7; males, 5; *, $P < 0.05$) at 7–9 months of age compared with age-matched wild-type mice (*n* = 15; females, 7; males, 8). Moreover, homozygous D187N mice were significantly worse at 12–14 months compared with homozygous D187N mice at 7–9 months, indicating a progressive impairment (##, $P < 0.01$; $F_{(1,58)} = 22.93$).

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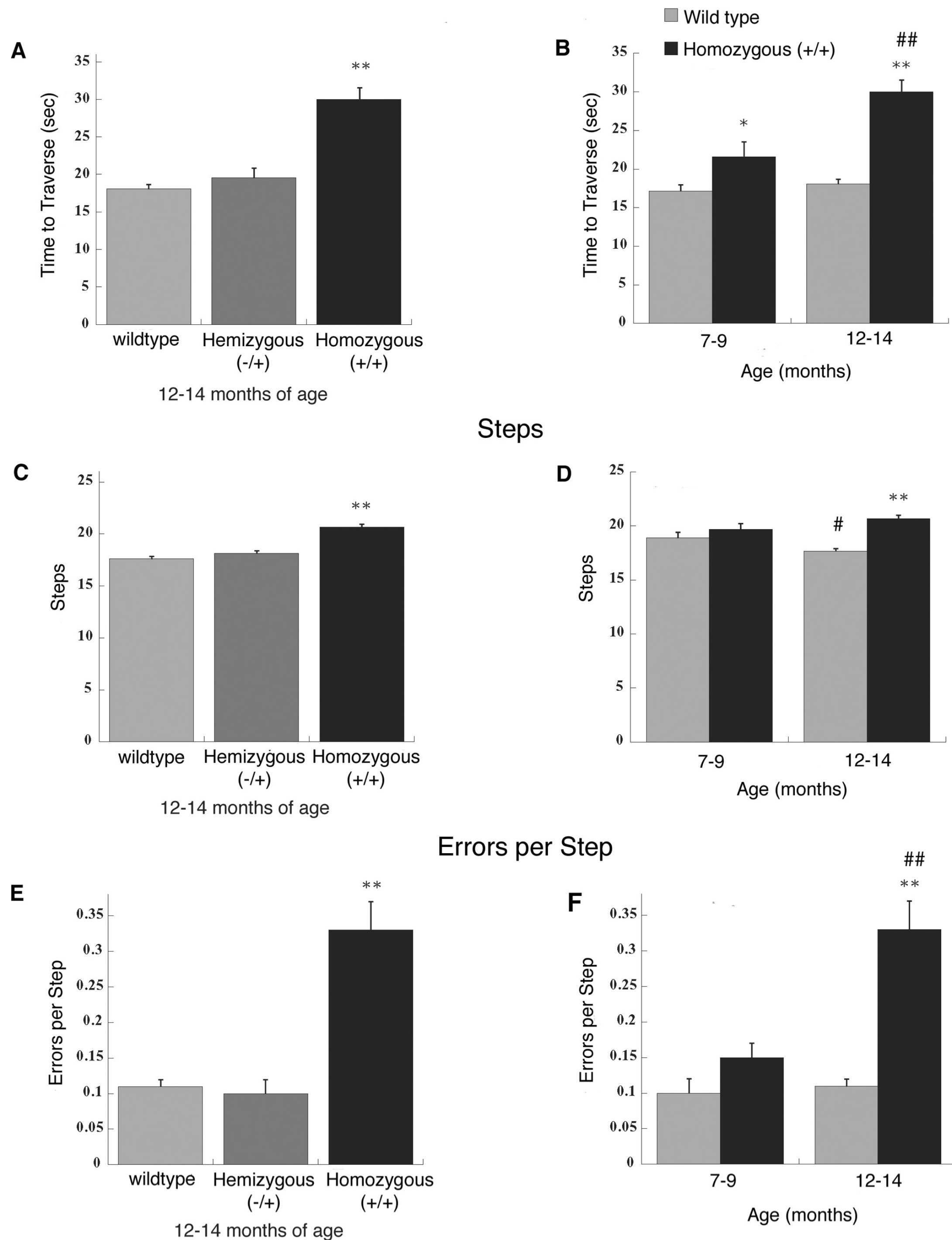

Fig. S5. Challenging beam traversal. Beam traversal was compared between wild-type ($n = 21$; females, 13; males, 8), hemizygous D187N (−/+) ($n = 20$; females, 10; males, 10), and homozygous D187N (+/+) ($n = 10$; females, 5; males, 5) mice at 12–14 months of age (*A*, *C*, and *E*). No significant difference between wild-type and hemizygous (−/+) mice was detected. However, time to traverse, number of steps, and errors per step for 12- to 14-month-old homozygous (+/+) mice was significantly worse than both wild-type and hemizygous mice (**, $P < 0.01$). (*B*) Time to traverse the beam was also significantly impaired in homozygous D187N (+/+) mice at 7–9 ($n = 12$; females, 7; males, 5) months of age compared with age-matched wild-type mice (7–9 months: $n = 15$; females, 7; males, 8) (*B*; *, $P <$ 05). Homozygous D187N mice were significantly worse at 12–14 months compared with homozygous D187N mice at 7–9 months in time to traverse and in errors per step indicating a progressive impairment (*B*, *F*; ##, $P < 0.01$). The number of steps in the 12- to 14-month wild-type mice is significantly less than in the 7- to 9-month wild-type mice (*D*, #) possibly because of their larger size.