

ErbB4 p.R927Q Mutant Mice Develop Features of Frontotemporal Dementia and ALS

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KEY TAKE AWAY: Preliminary phenotypic analyses of mice bearing the familial *ERBB4* p.R927Q mutation indicate that it is a driver of FTLD and ALS.

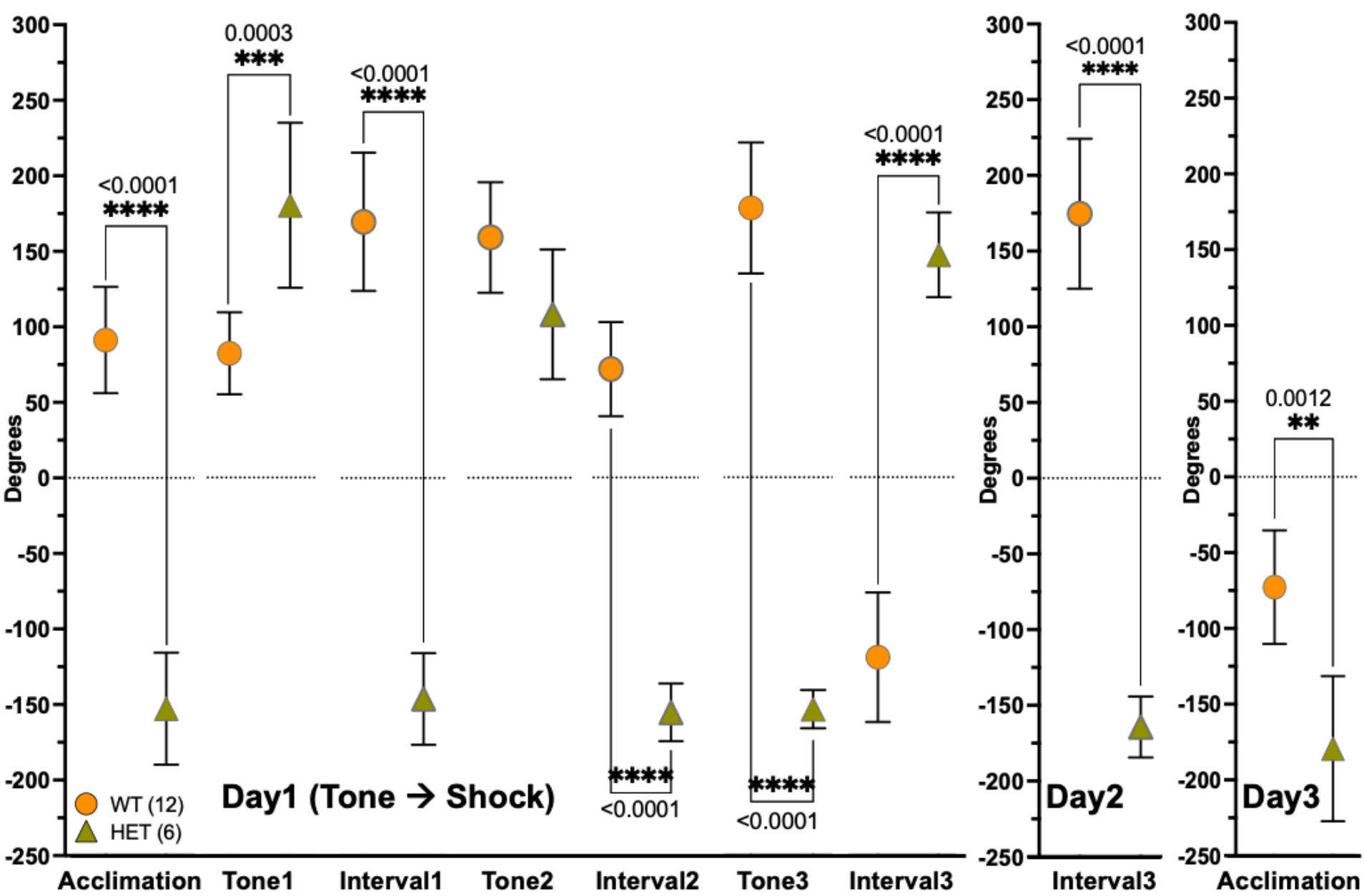
INTRODUCTION

Frontotemporal dementia (FTD) involves progressive deterioration of behavior, executive function, trait and personality and is evident structurally as frontotemporal lobar degeneration (FTLD). FTLD with pathological TDP43 neural/glial inclusions (FTLD-TDP), often coexists with TDP43-positive amyotrophic lateral sclerosis (ALS). Several families have been identified that inherit autosomal dominant FTLD/ALS or ALS alone and mutations in the gene encoding *ERBB4* receptor tyrosine kinase (RTK). Although the neuropathology associated with *ERBB4* mutations remain obscure, *ERBB4* p.R927Q or p.I712M mutations show reduced phosphorylation when stimulated with the *ERBB4* ligand neuregulin-1 β , suggesting that reduced *ERBB4* activity causes FTLD/ALS or ALS. Here, we seek to determine whether these mutations are drivers of this neuropathology.

METHODS

We generated mice carrying p.I712M (familial FTLD/ALS) or p.R927Q (familial ALS only) *ErbB4* mutations. Behavioral studies were performed using Barnes Maze (BM) and Open Field Assays (OFA); gait and mobility were assessed with CatWalk (CW); and body composition was measured by DXA scans in *ErbB4*-R927Q mutants and wild-type (WT) littermates. At two years of age, multiple organs and tissues were collected for histological imaging and molecular analyses. Plasma was obtained for biomarker studies using QuanterixTM Simoa-assays. FLIPR ion flux assays were conducted on cortical and hippocampal neurons from mutant and WT mouse embryos and on differentiated motor neurons from WT and mutant human stem cells.

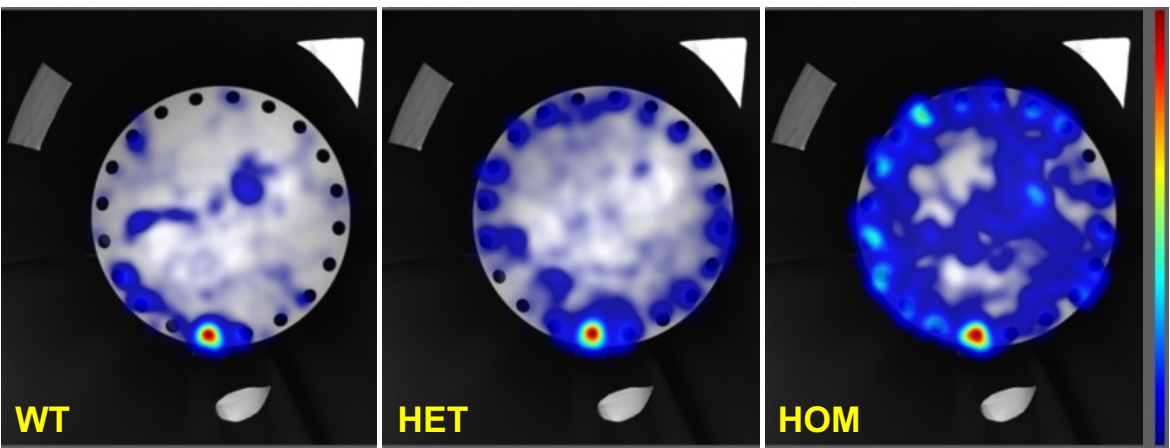
RESULTS



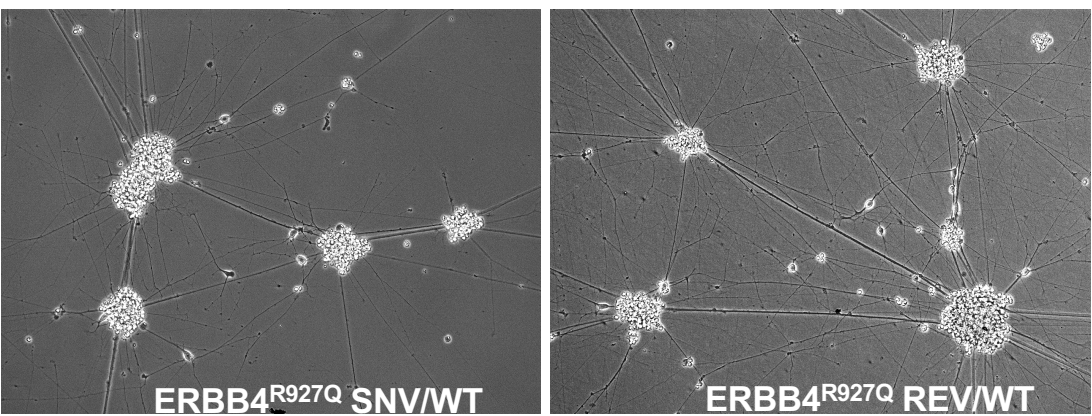
Fear Conditioning (FC) of 18-Month-Old *ErbB4*^{R927Q} Mutants. Among multiple parameters tested (e.g. activity state, freezing, cumulative freezing, etc.), ‘Heading’ produced the most significant results (shown). Heading indicates directional change of movement from frame to frame (30 frames/s). On Day1, HETs were quite distinct in their Heading compared to WT in most stages of FC. Likewise, on the final Interval Day2, and Acclimation Day3, the HETs maintained distinction from the WT. Open Field experiments produced the same results (not shown). Collectively, these data suggest the R927Q allele in mice and possibly in patients, impacts motor skills, anxiety and/or memory/learning phenotype. Means, SEMs and significance using Two-Way ANOVA with Tukey’s multiplicity correction.

F8ic totals (132): 39 WT : 63 HET : 30 HOM = 1.00 : 1.62 : 0.77
Males (68): 24 WT : 30 HET : 14 HOM = 1.00 : 1.25 : 0.58
Females (64): 15 WT : 33 HET : 16 HOM = 1.00 : 2.20 : 1.07

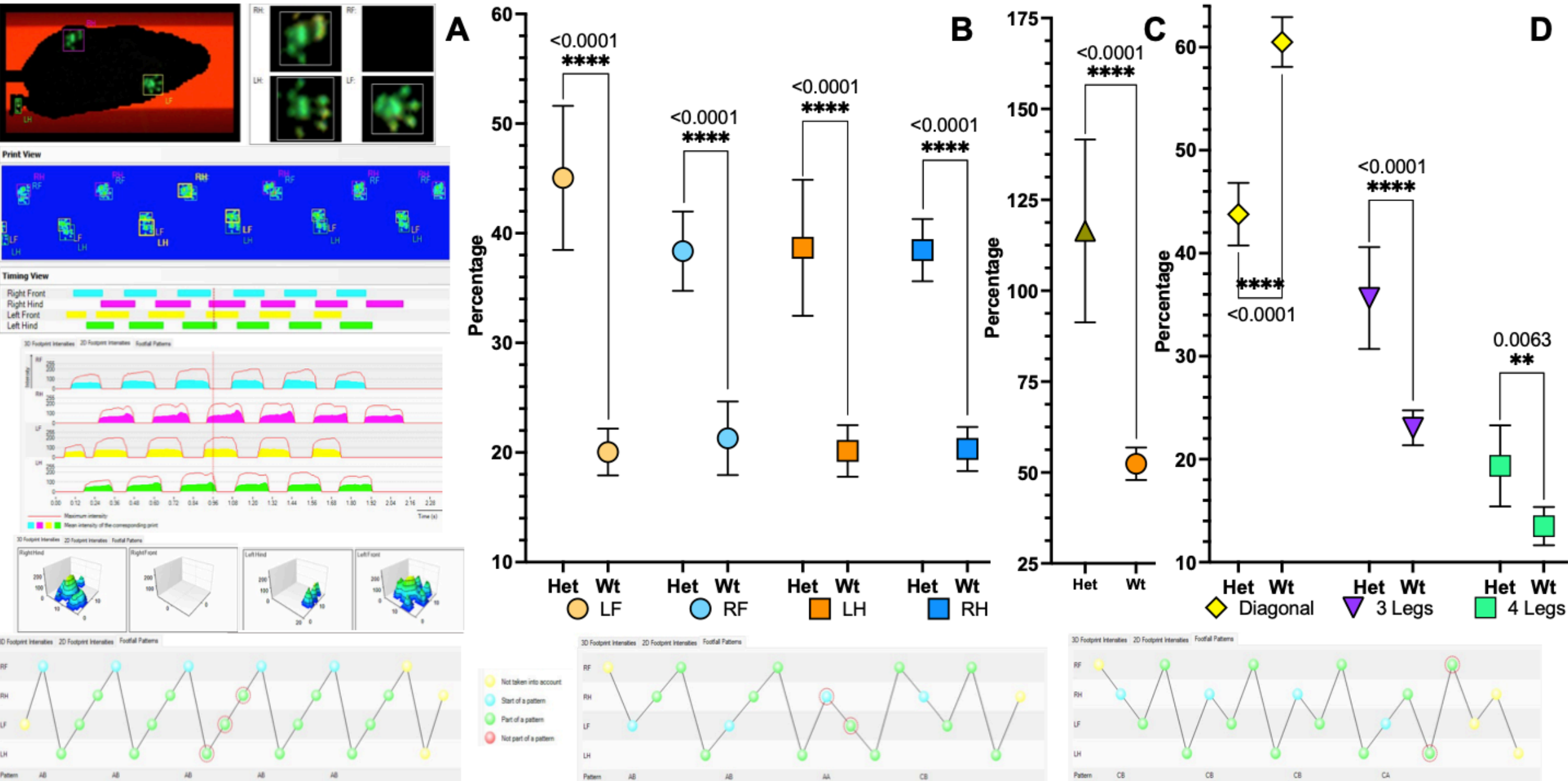
Non-Mendelian ratios in *ERBB4*^{R927Q} Mutant Male Mice. Reduced numbers of viable heterozygous (HET) and Homozygous (HOM) male pups were observed from intercrosses between F8 backcross matings (F8ic). As shown, female pup numbers were unaffected (1:2:1 ratios).



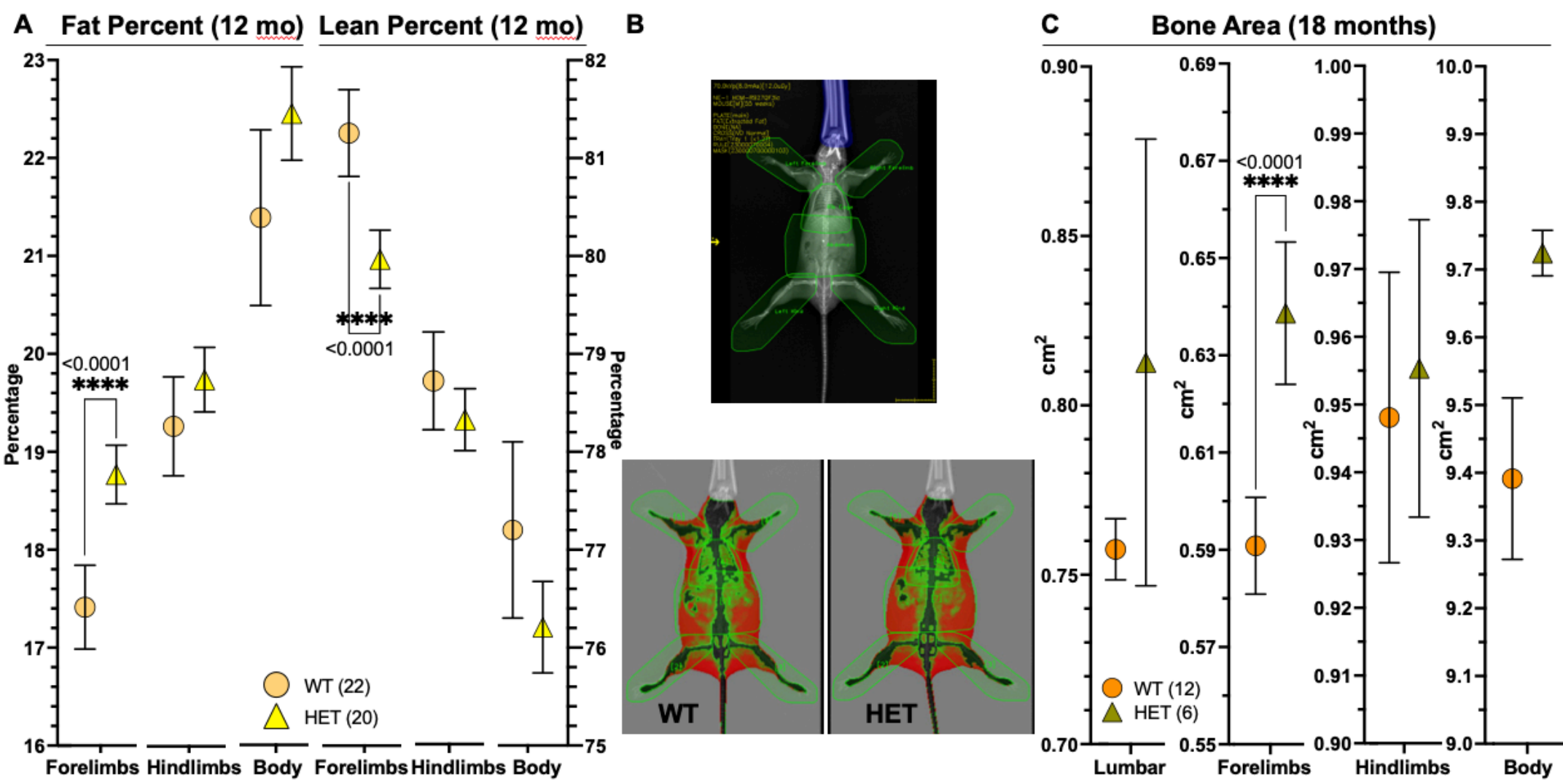
Barnes Maze Shows Learning/Memory and Anxiety Phenotypes in 12-Month-Old *ErbB4*-R927Q males. Heatmaps show grouped averages at testing after habituation and movement of goal box (GB; Red). Heterozygotes (HET) and homozygotes (HOM) take progressively longer finding the GB than wild-type (WT) littermates, with HETs mostly staying to the periphery.



Human iPSC-Derived *ERBB4*^{R927Q} Motor Neurons Show Decrease Neurite Outgrowth and Branching. Human iPSCs were subjected to a 32-day in vitro motor neuron differentiation protocol (Workman, et al, 2023). HET motor neurons produced substantially fewer neurite outgrowths and reduced branching compared to WT.



Altered Gait & Mobility in 18-Month *ErbB4*^{R927Q} Males. **A) CatWalk-XT** visualization of footfall patterns, 2D/3D mean/max intensities, and three exemplary footfall sequences. **B) Limb Speed Variation** and **C) Walking Speed Variation** show HET males display increases in variability. **D) Standing Support** shows that HET males prefer standing on 3 or 4 legs compared to WT, indicative of decreased balance. Six compliant walks per male were classified and averaged for data analysis (Mean, SEM data N=10 mice per group; Two-Way ANOVA with Tukey’s multiplicity correction). Heterozygote (HET), Wild type (WT), Left Front (LF), Right Front (RF), Left Hind (LH) Right Hind (RH). Other significant data was also observed (not shown).



DXA Scans on 12- and 18-Month *ErbB4*^{R927Q} Mutants. **A)** At 12-months of age, fat percentages were the highest in the HETs compared to WT, suggesting a dominant effect of the mutation in males. Significant differences were found in the Forelimb region of interest (ROI) compared to others. Similarly, lean percentages were the lowest in the HETs, with significant differences found in the Forelimb ROI. **B)** OsteoSys iNSiGHT images of 12-month-old males exemplifying Fat (red), Lean (green) and Bone (grey) measurements. Total weights followed fat percentages. **C)** At 18-months of age, bone areas were the highest in the HETs, with significant differences found in the Forelimb ROI. Of note, the highest bone areas corresponded to the lowest bone mineral densities (data not shown). Means, SEMs and significance using Two-Way ANOVA with Tukey’s multiplicity correction. Wild type (WT) and heterozygous (HET) numbers are shown.

CONCLUSIONS

Findings suggest that the *ErbB4*-R927Q mutation contributes to FTLD and ALS phenotypes. Ongoing studies include expanded behavioral testing across additional animals and ages, and analyses of *ErbB4*-I712M mutants. Neuropathological assessments are in progress, including stereology and morphometry to evaluate the loss of *ErbB4*-expressing inhibitory interneurons and motor neurons, synaptic loss, and the temporal progression of these changes.

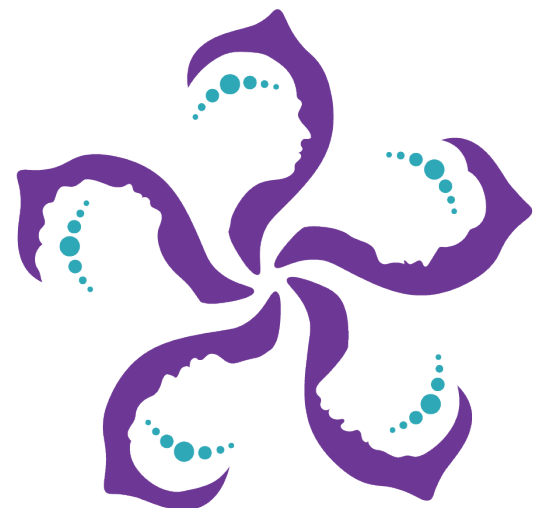
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Please also see abstract #10206 “Changes in Neurodegenerative Biomarkers Following Computerized Cognitive Training: Findings from the ENACT Study.” Hye Won Chai, *et al.*

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