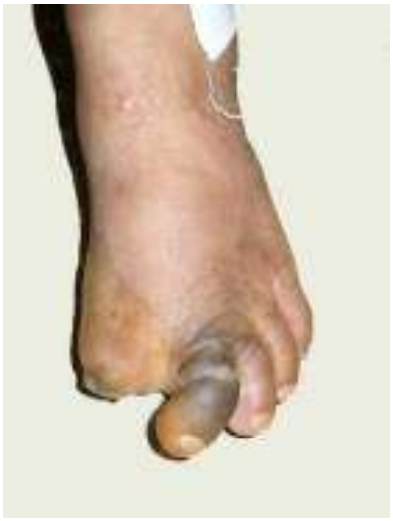
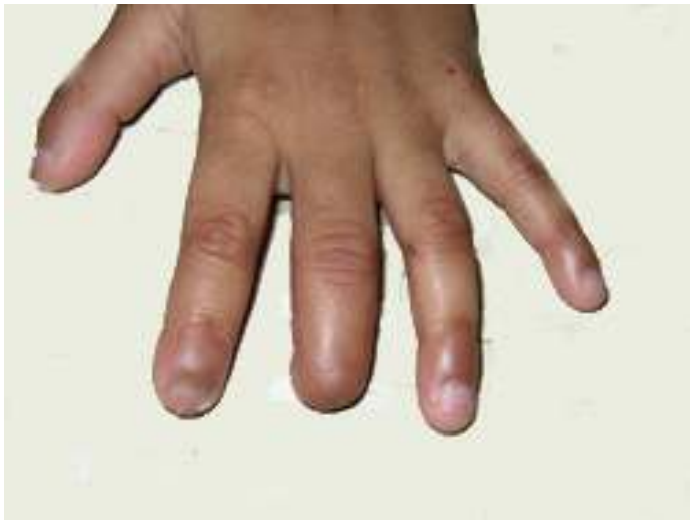
Overexpression of serine palmitoyltransferase rescues the phenotype of hereditary sensory and autonomic neuropathy

hereditary sensory and autonomic neuropathy (type I)





autosomal dominant

characterized by a sensory deficit in the distal portion of the lower extremities, chronic perforating ulcerations of the feet and progressive destruction of underlying bones.

missense mutations in SPTLC1

⇓

enzyme serine palmitoyltransferase

⇓

hereditary sensory and autonomic neuropathy (type I)

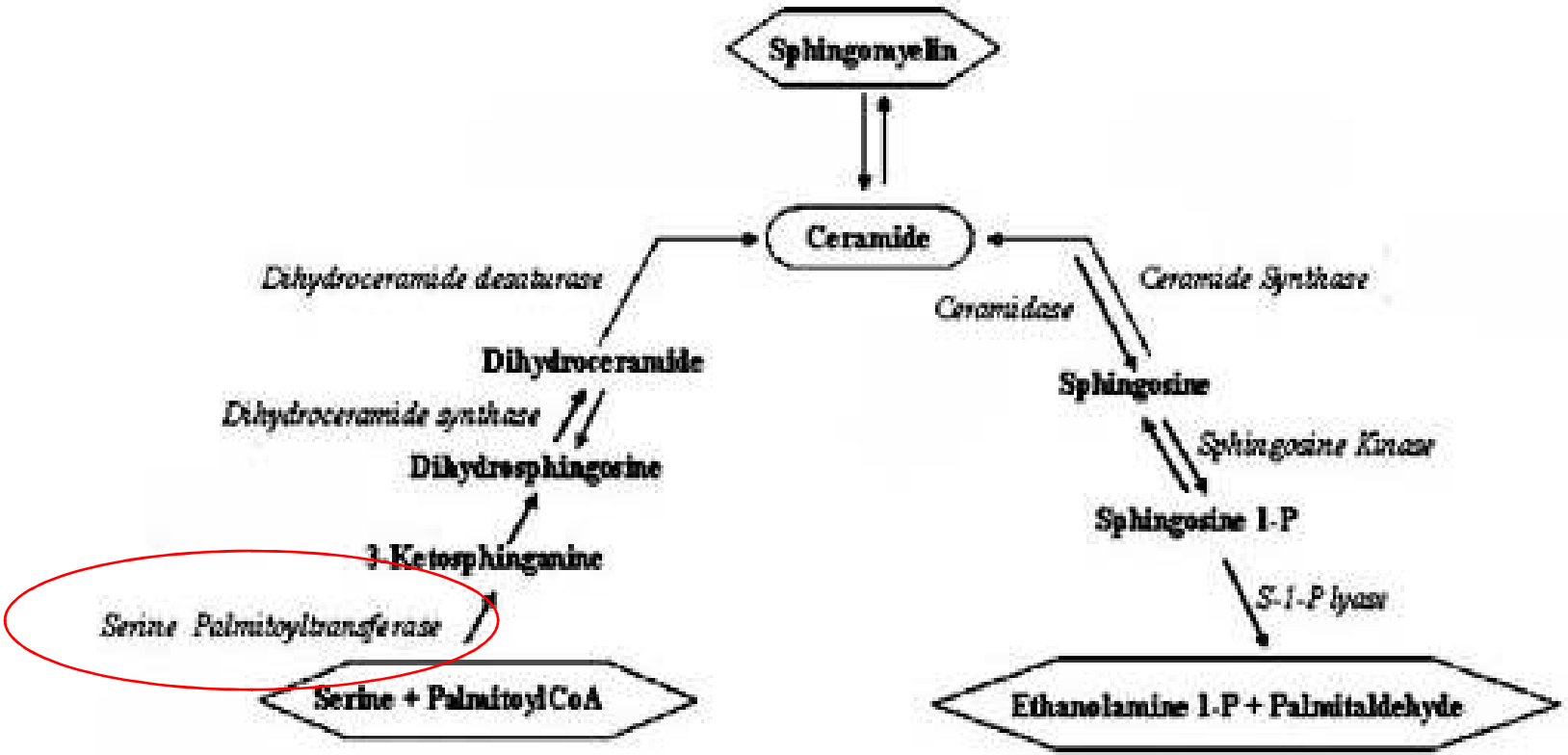
missense mutations in SPTLC1

⇓

enzyme serine palmitoyltransferase

⇓

hereditary sensory and autonomic neuropathy (type I)



hereditary sensory and autonomic neuropathy (type I) - HSAN1

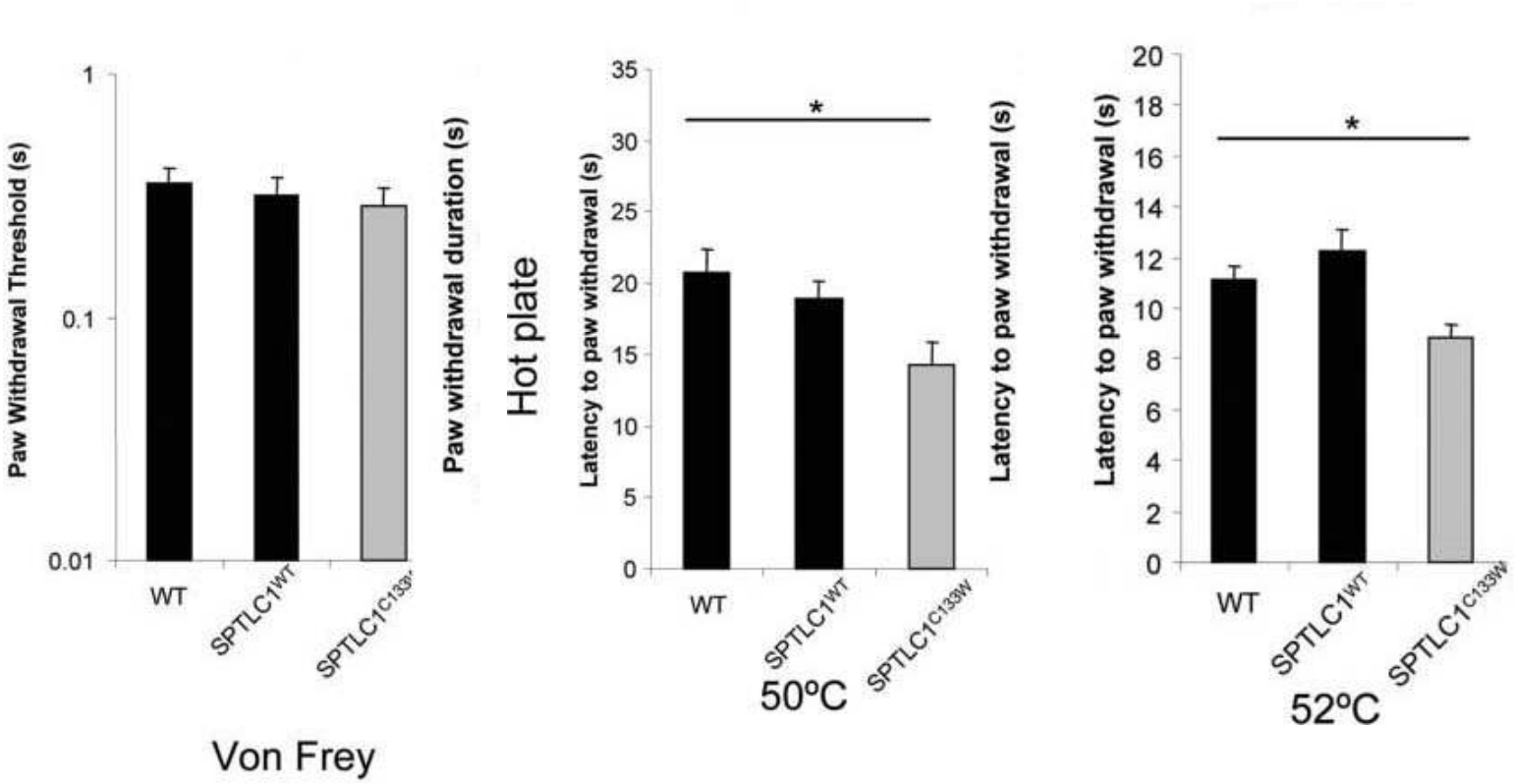
- AD inheritance pattern
- age of onset
- type of mutation

2 hypotheses: **loss- or gain of function**

generated transgenic mouse lines that ubiquitously overexpress :

wild-type(SPTLC1WT) or
mutant SPTLC (SPTLC1C133W)

as the SPTLC1C133W mice age, they develop evidence of a loss-of-function, small fiber sensory neuropathy.



10 months: evidence of hyperpathia

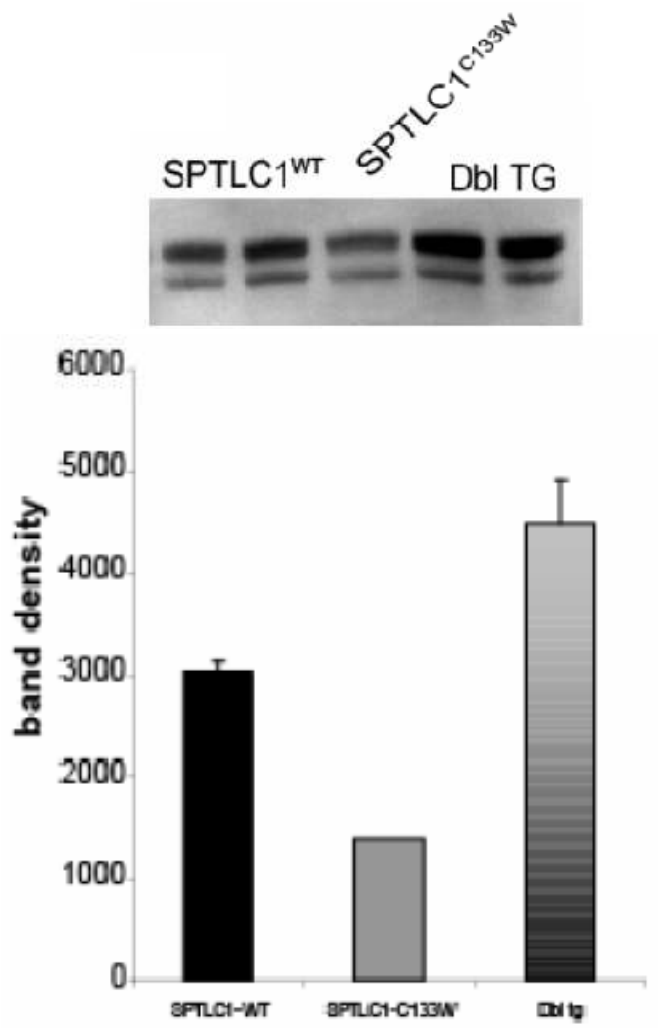
(McCampbell, Human Molecular Genetics, 2005)

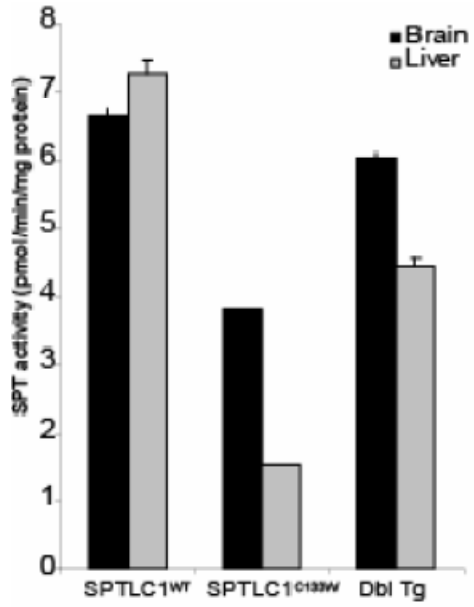
In order to determine whether the phenotype was the result of a toxic mutant protein or the loss of SPT activity we crossed the **wildtype overexpressing** line (6F) with the **C133W overexpressing** line (8E)

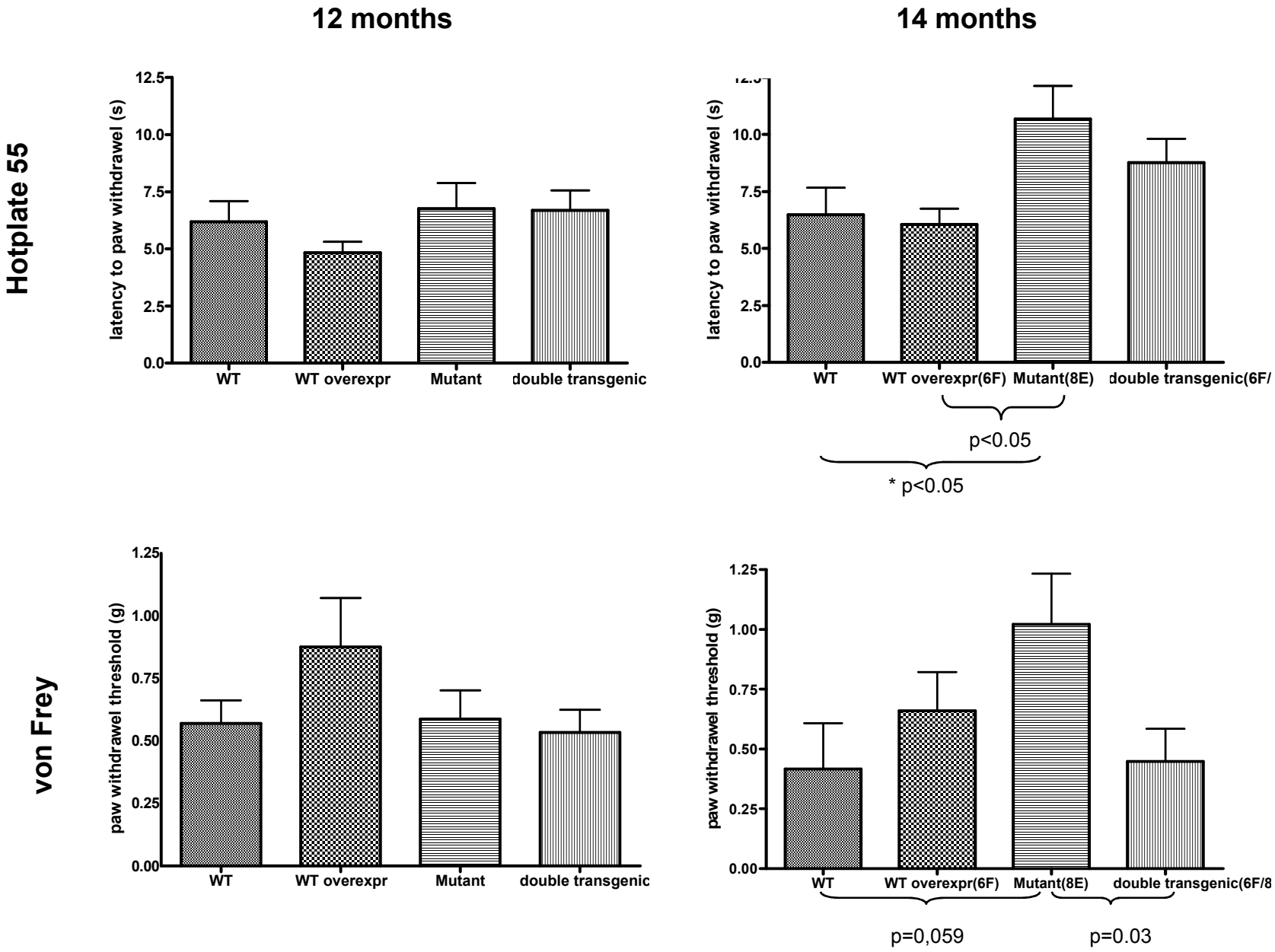
⇓

double transgenic mice that overexpress both the wild-type and mutant SPTLC1

Normalization of SPT activity in double transgenic mice






12 months
14 months
Hotplate 55
von Frey
latency to paw withdrawel (s)
paw withdrawel threshold (g)
WT
WT overexpr
Mutant
double transgenic
WT overexpr(6F)
Mutant(8E)
double transgenic(6F/
double transgenic(6F/8
p<0.05
* p<0.05
p=0,059
p=0.03

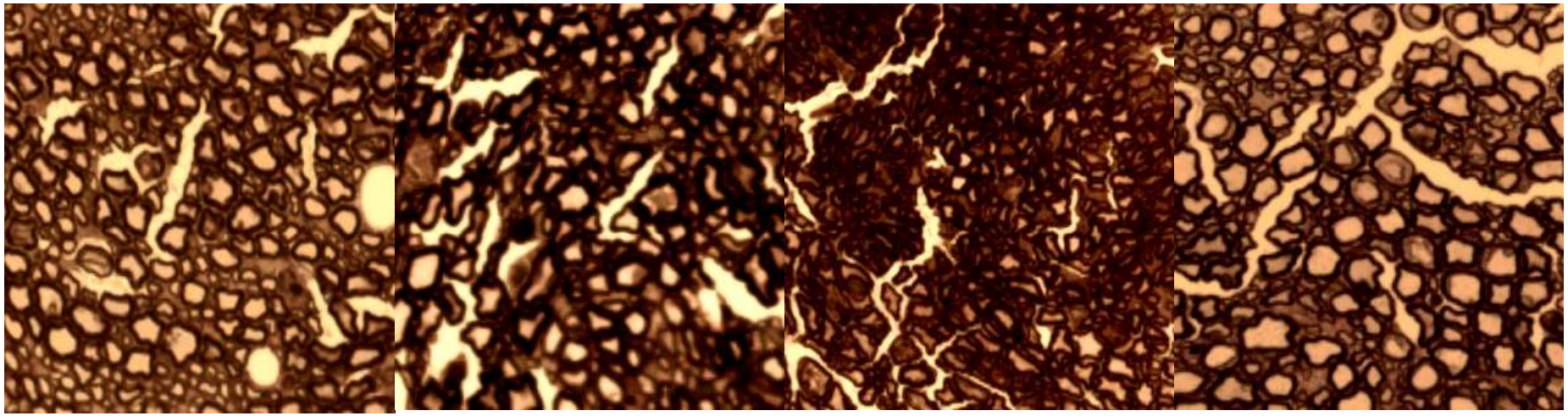
Sensory Performance

Evaluated in 12 and 14 month old WT, SPTLC1WT, SPTLC1C133W and double transgenic mice with Von Frey hair, pin prick assay, acetone exposure and hot plate test.

14-month-old SPTLC1C133W mice were significantly less sensitive to mechanical stimuli and slower to react in the hot plate tests at 55^{o}C ($p < 0.05$).

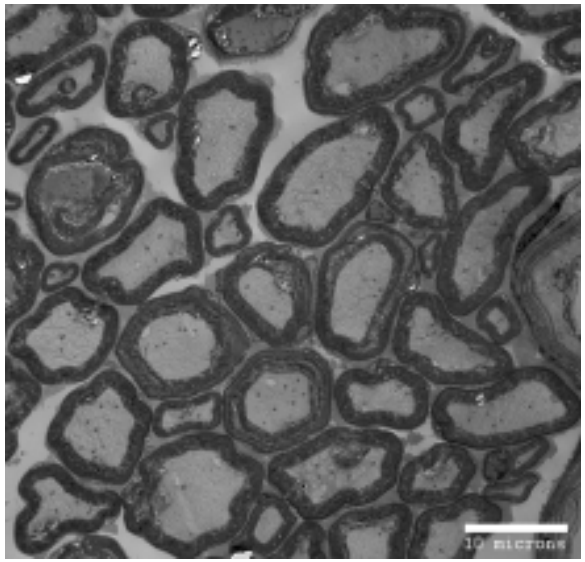
At 14 months, statistically significant improvements on sensory testing are seen in the double transgenic animals

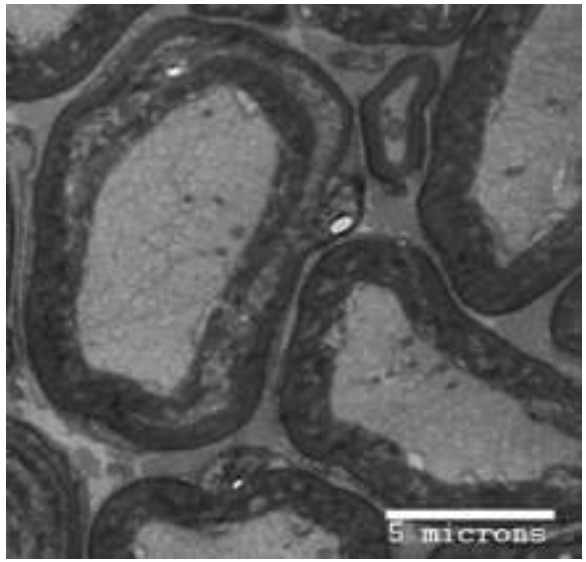
Sciatic nerve



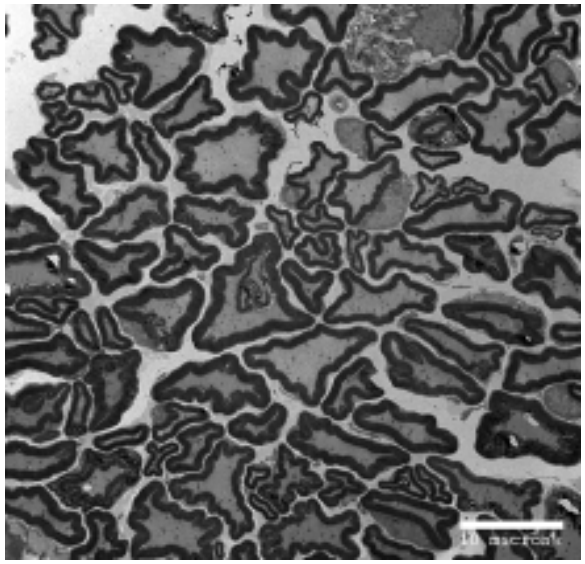
WT WT overexpresser Mutant double transgenic

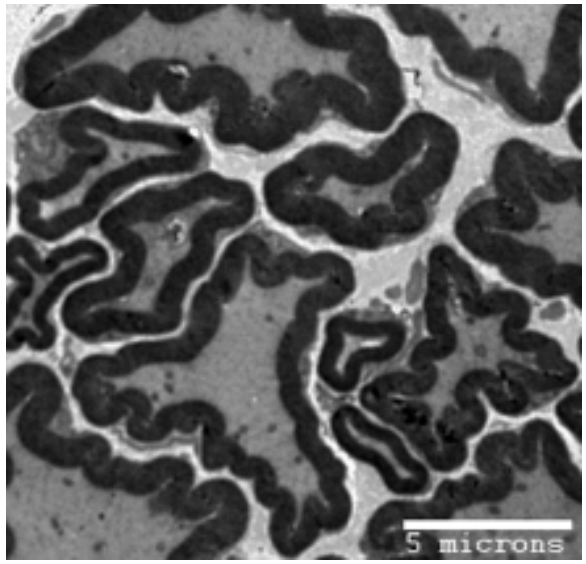
wildtype



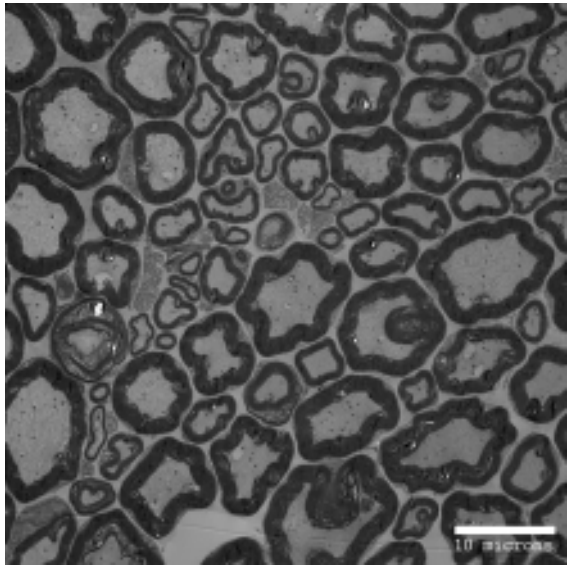


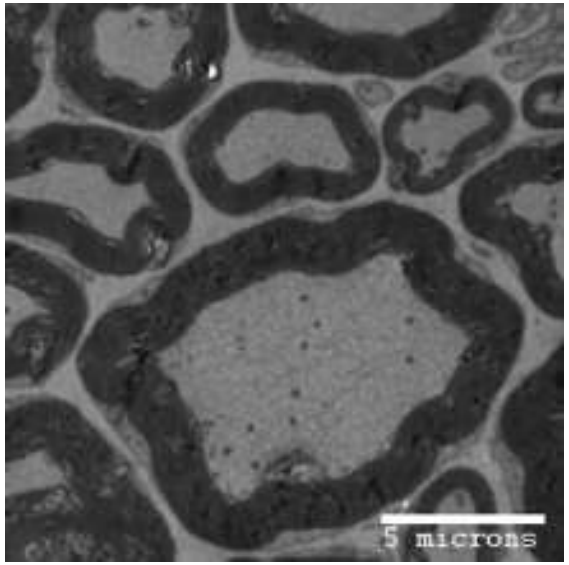
SPTLC1 mutant



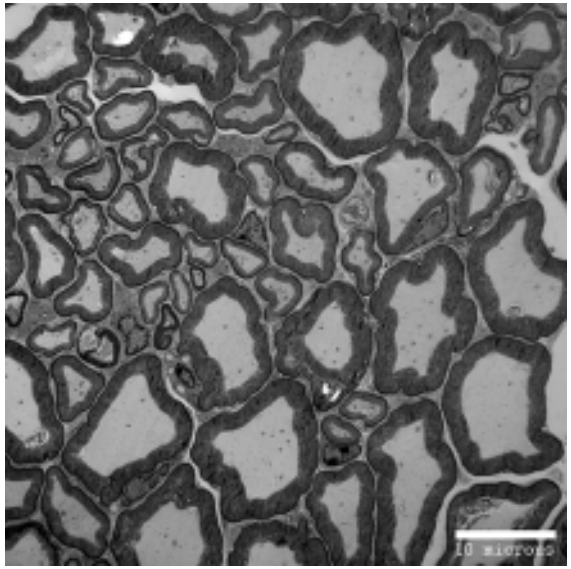


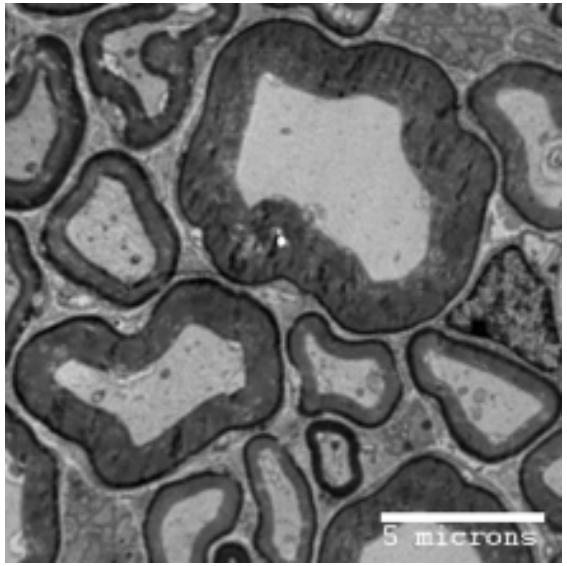
wildtype overexpresser





double transgenic





Preliminary Pathology

In the sciatic nerves:

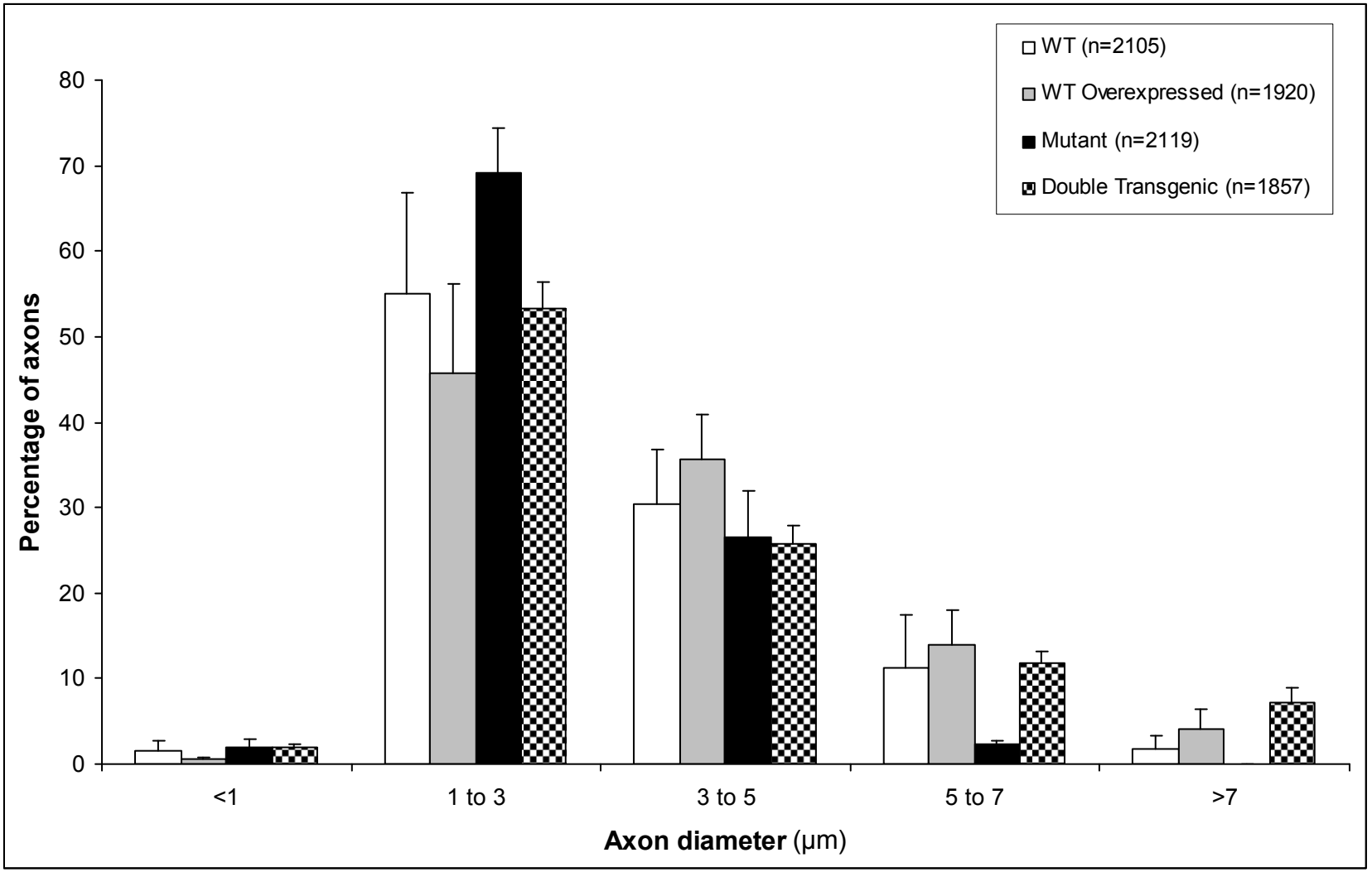
the 8E mutants with marked loss of fibers, and a shift toward less large fibers and more small fibers

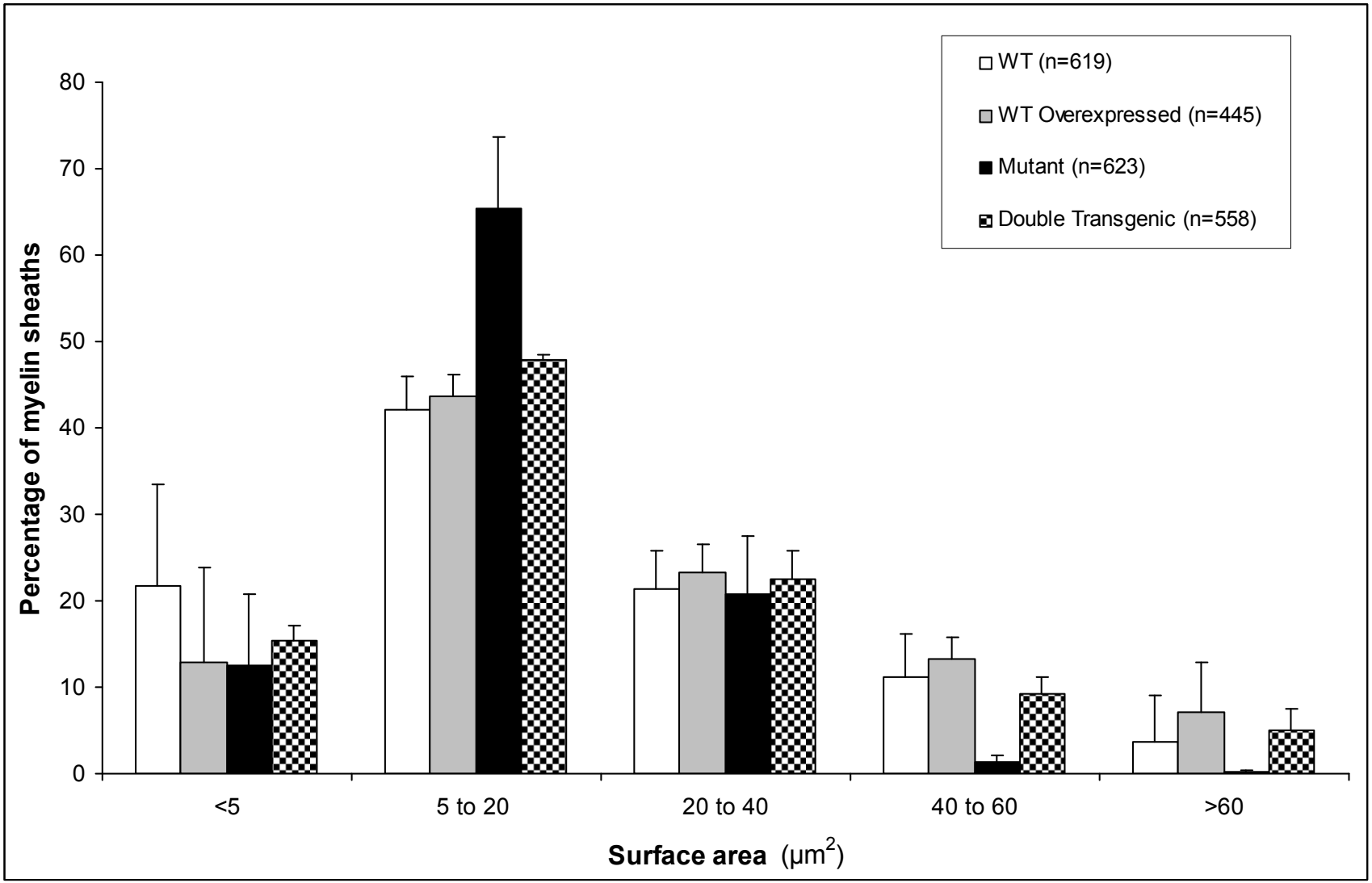
In the roots:

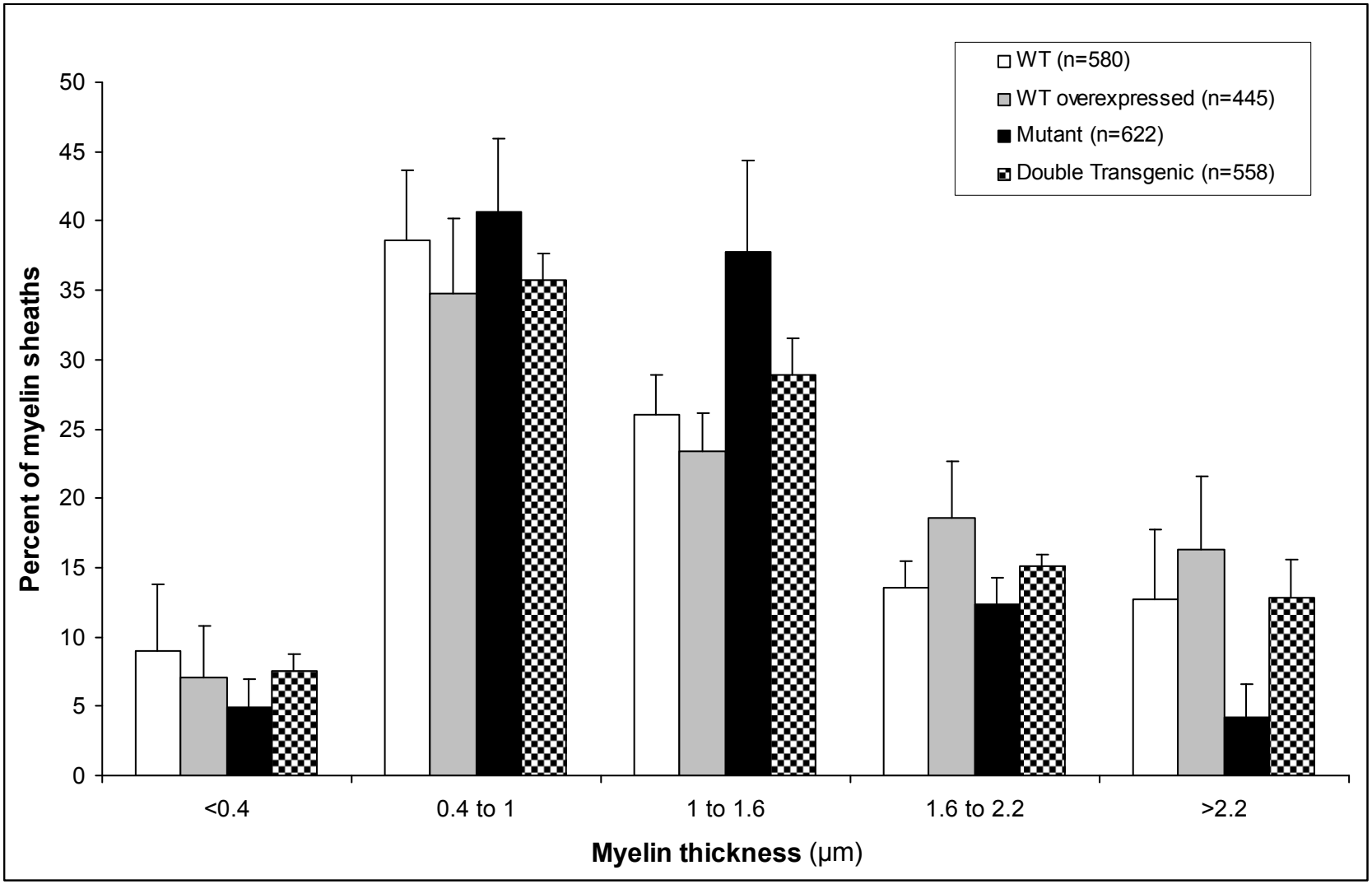
the 8E mutants with marked thinning of myelin especially around the large fibers; the thickness of myelin around the large fibers resembles that of the smaller fibers

double transgenics: absence of abnormal pathology

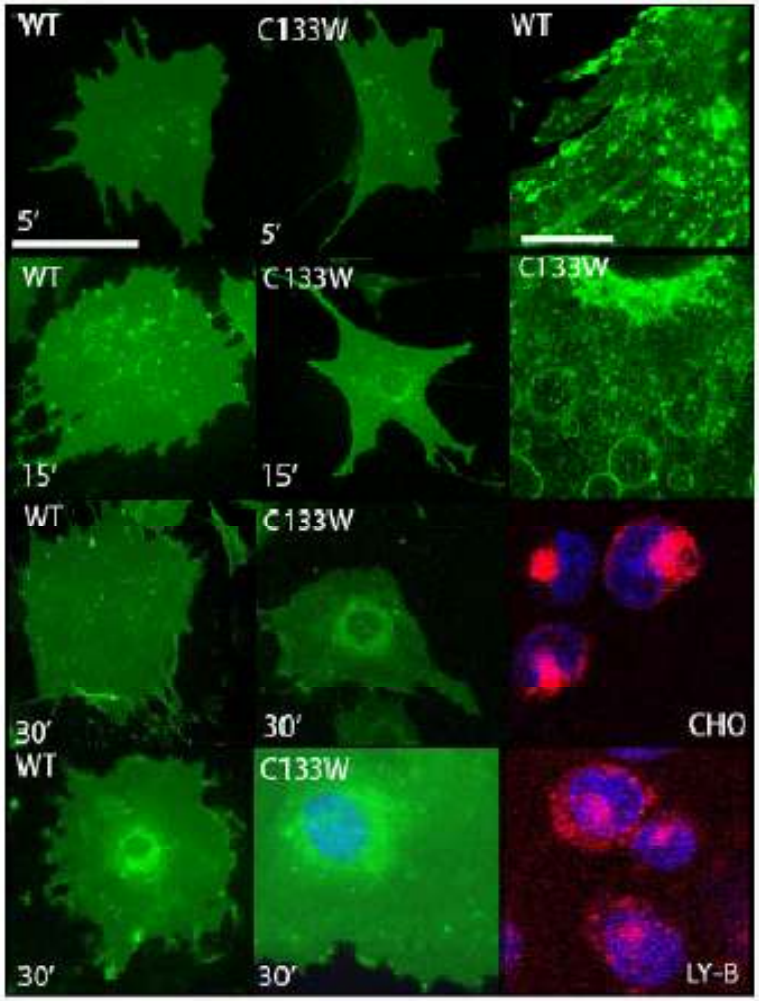
but ? loss of small fiber axons, EM pending.


WT (n=2105)
WT Overexpressed (n=1920)
Mutant (n=2119)
Double Transgenic (n=1857)
Percentage of axons
80
70
60
50
40
30
20
10
0
<1
1 to 3
3 to 5
5 to 7
>7
Axon diameter (µm)

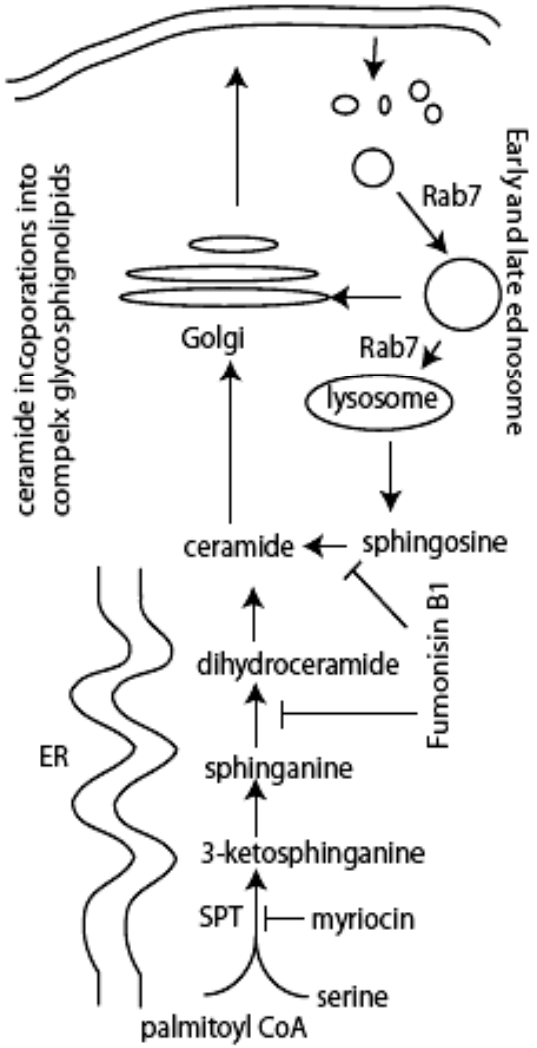

WT (n=619)
WT Overexpressed (n=445)
Mutant (n=623)
Double Transgenic (n=558)
Percentage of myelin sheaths
80
70
60
50
40
30
20
10
0
<5
5 to 20
20 to 40
40 to 60
>60
Surface area (µm2)

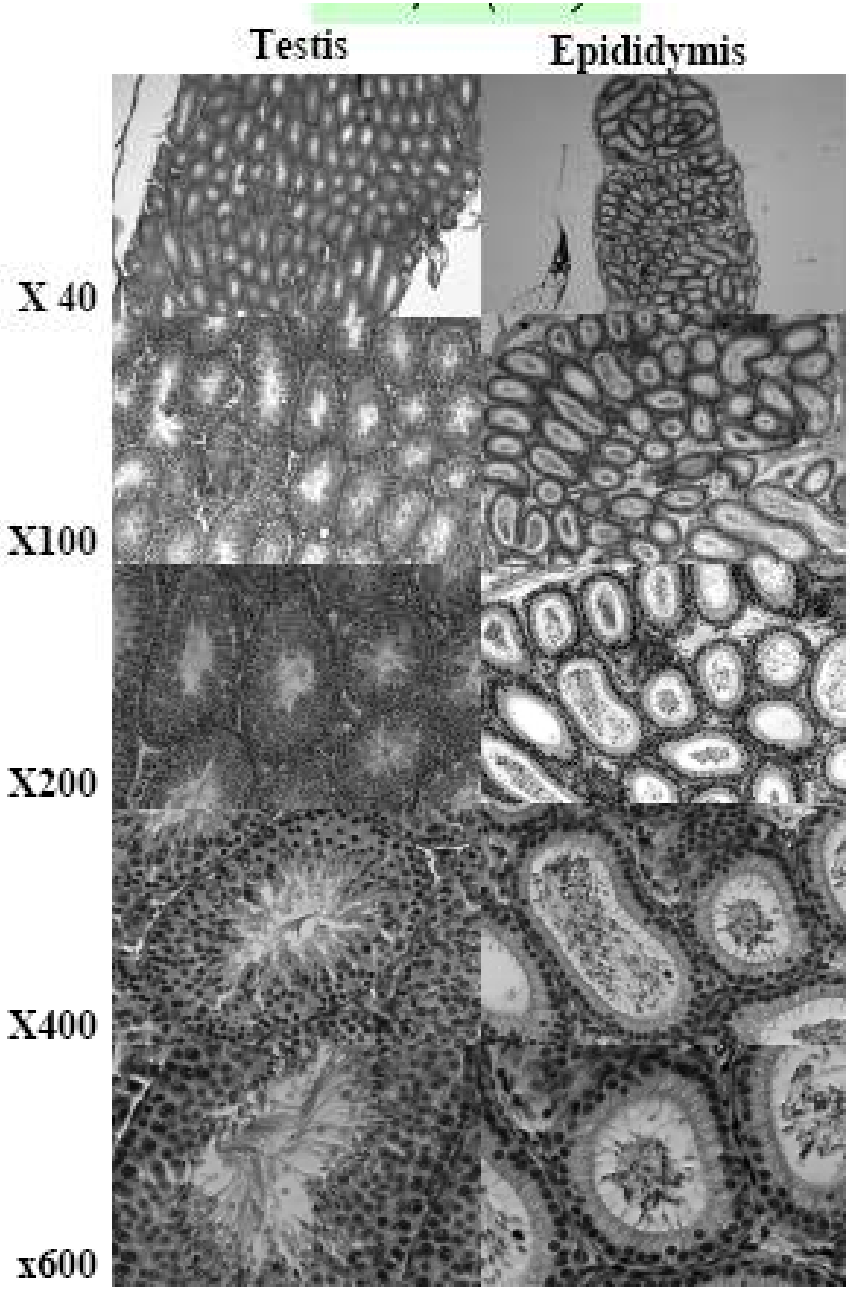

WT (n=580)
WT overexpressed (n=445)
Mutant (n=622)
Double Transgenic (n=558)
Percent of myelin sheaths
50
45
40
35
30
25
20
15
10
5
0
<0.4
0.4 to 1
1 to 1.6
1.6 to 2.2
>2.2
Myelin thickness (µm)

Mutant SPT alters endocytic trafficking



Major metabolic and trafficking pathways for sphingolipids



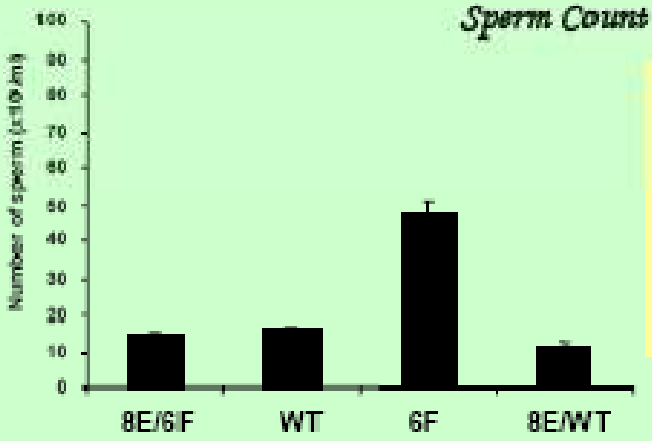

Testis
Epididymis
X 40
X100
X200
X400
x600

Analysis of the Male Reproductive Organ

Xin-Yuan Lee, M.D.

Sperm Analysis

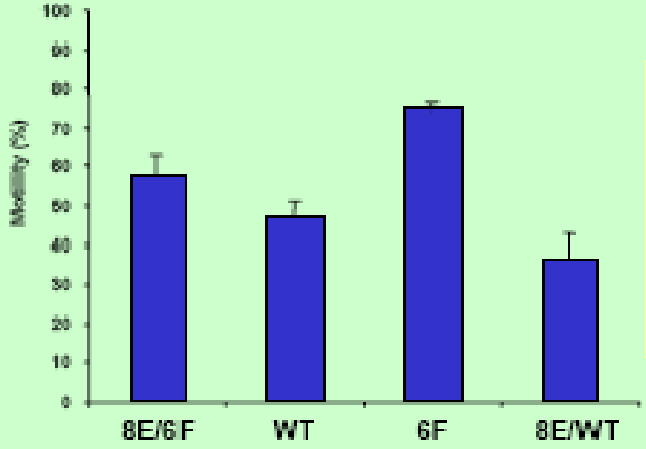
Sperm Count



Group	Sperm Number (x10⁶/ml)
8E6F	14.50 ± 0.89
WT	16.17 ± 1.01
6F	47.50 ± 3.49
8EWT	11.33 ± 1.31

mean ± S.E.

Sperm Motility



Group	Motility (%)
8E6F	57.47 ± 5.38
WT	47.35 ± 4.04
6F	74.46 ± 1.95
8EWT	36.47 ± 6.24

mean ± S.E.

Testis weight

Group	8E / 6F	WT	6F	8E / WT
Weight(mg)	81.5	80.1	74.0	61.3

Calendar

- paper submission
- RO1 deadline
- Glycolipid and Sphingolipid meeting 2/17/08
- HSAN1 meeting Boston

Conclusions

- neuropathy arises from loss of SPT function, rather than from a novel, adverse effect of the mutant SPTLC1C133W mutant protein

- supplementation of serine palmitoyltransferase activity in the mice (adding the normal SPTWT transgene) reverses the clinicopathological phenotype