A journey of peripheral neuropathy: from neurodegeneration and pain to amyloidosis

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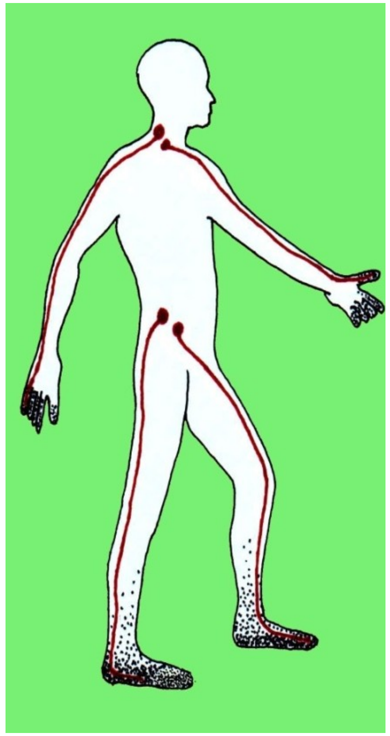
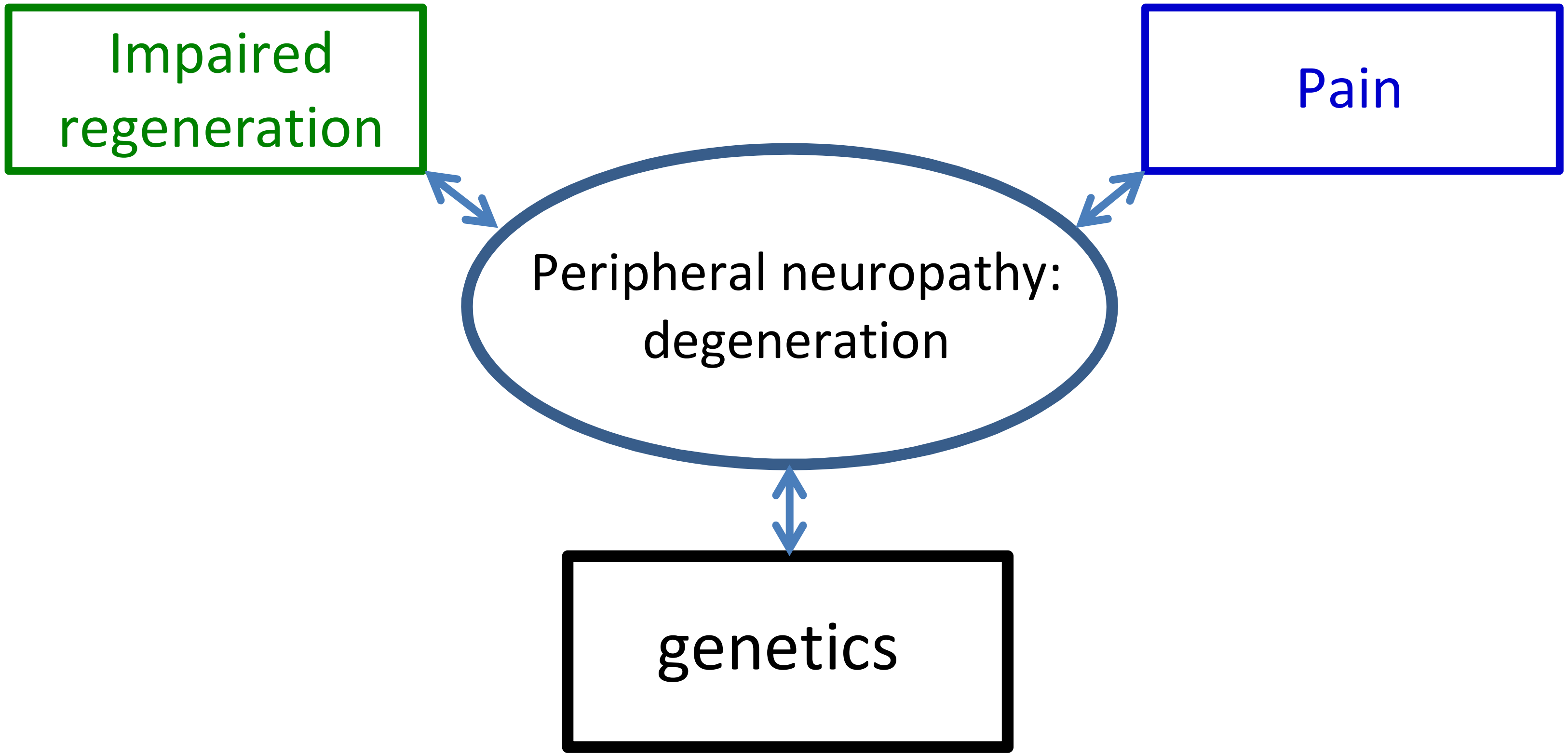
Peripheral Neuropathy

- 45 y/o male with diabetes for 5 years (HbA1c = 7.2%)
- burning, tingling, and electric-shock sensation over both feet and hands; but skin temperature was normal.
- painless wounds over limbs with reduced pain and temperature sensations.
- Nerve conduction studies: Normal.
- Physicians and this patient were puzzled.



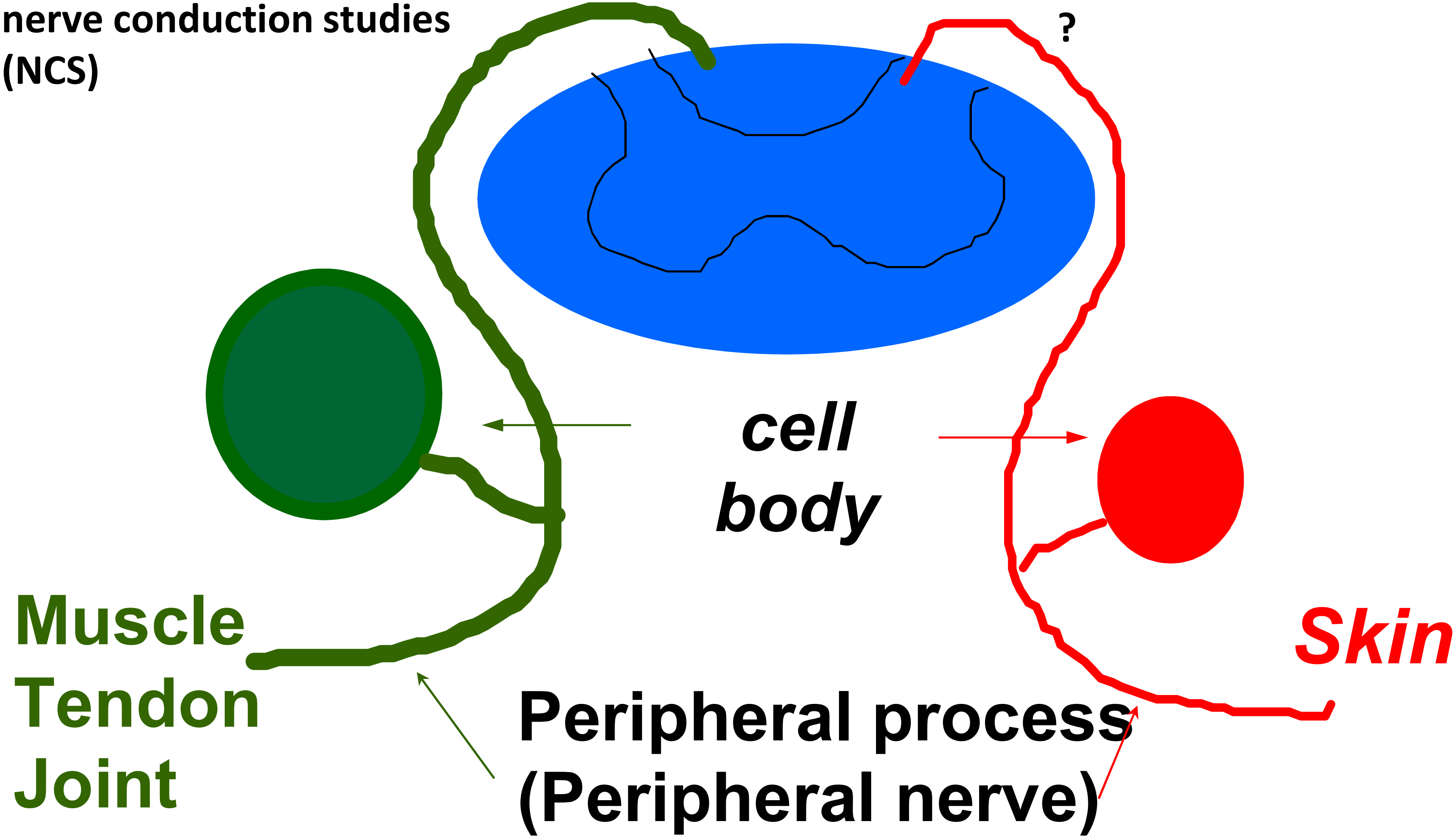
Small fiber neuropathy: degeneration and pain



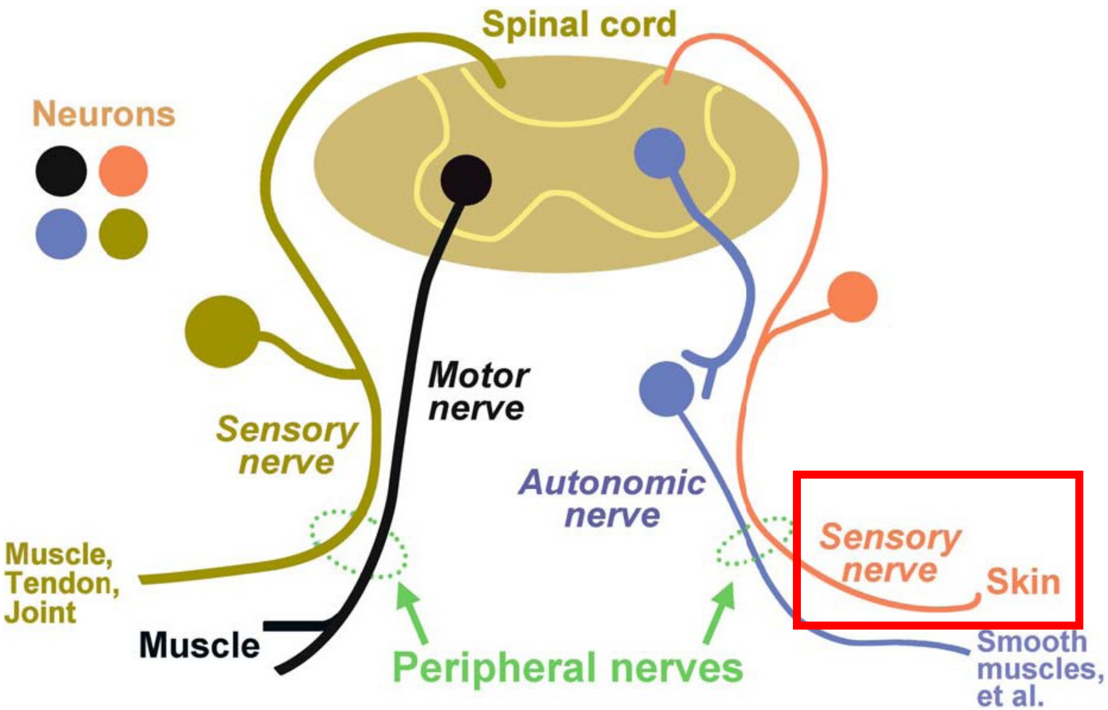
Sensory neuron

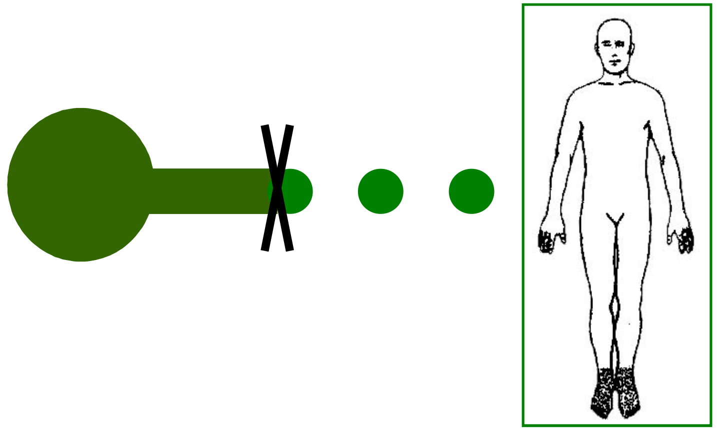
<u>Large fiber neuropathy</u>
nerve conduction studies (NCS)

<u>Small fiber neuropathy</u>
?



Skin biopsy for small fiber neuropathy





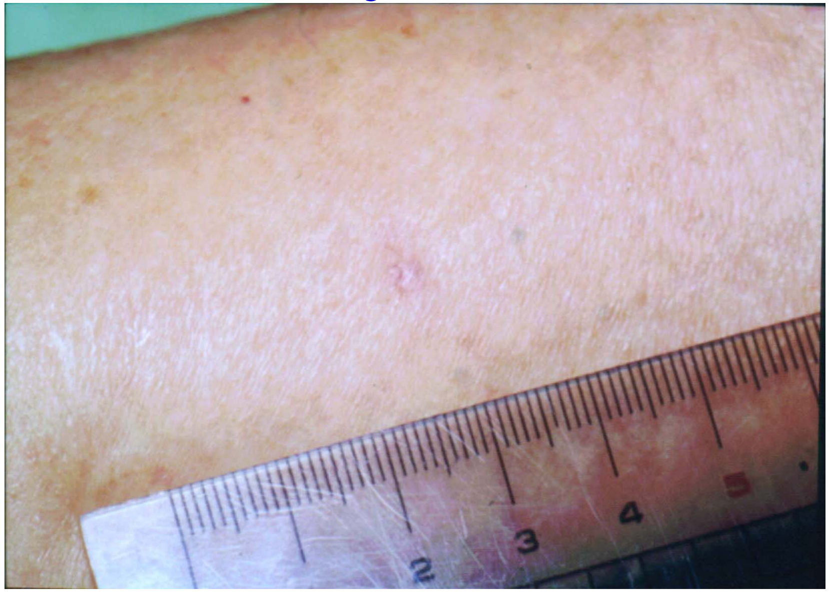
- Dying-back neuropathology in Diabetic neuropathy: length-dependent neuropathy
- Skin biopsy for “free nerve ending”

(Hsieh ST, from Encyclopedia of Pain,

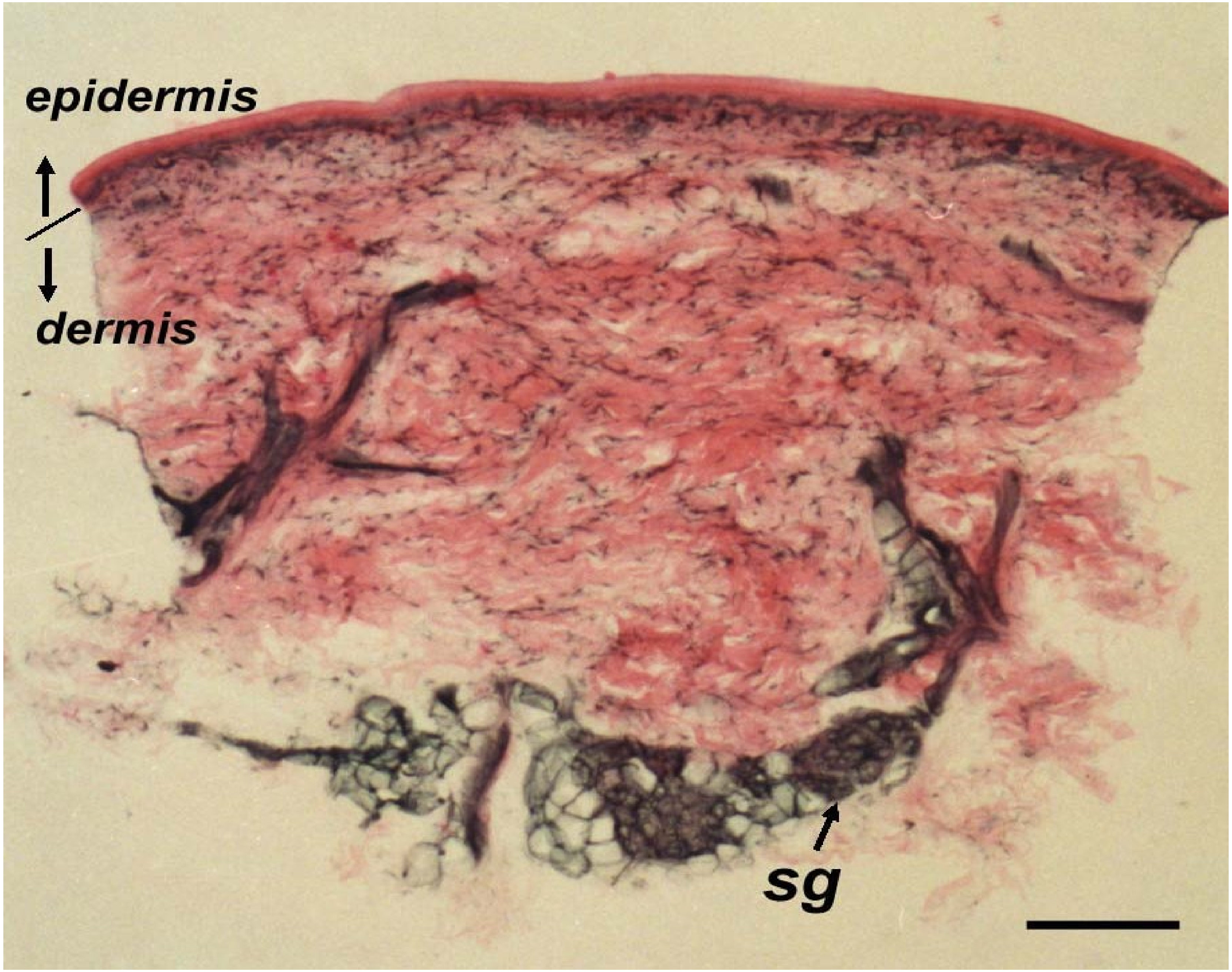
3 mm punch skin biopsy

2 weeks later

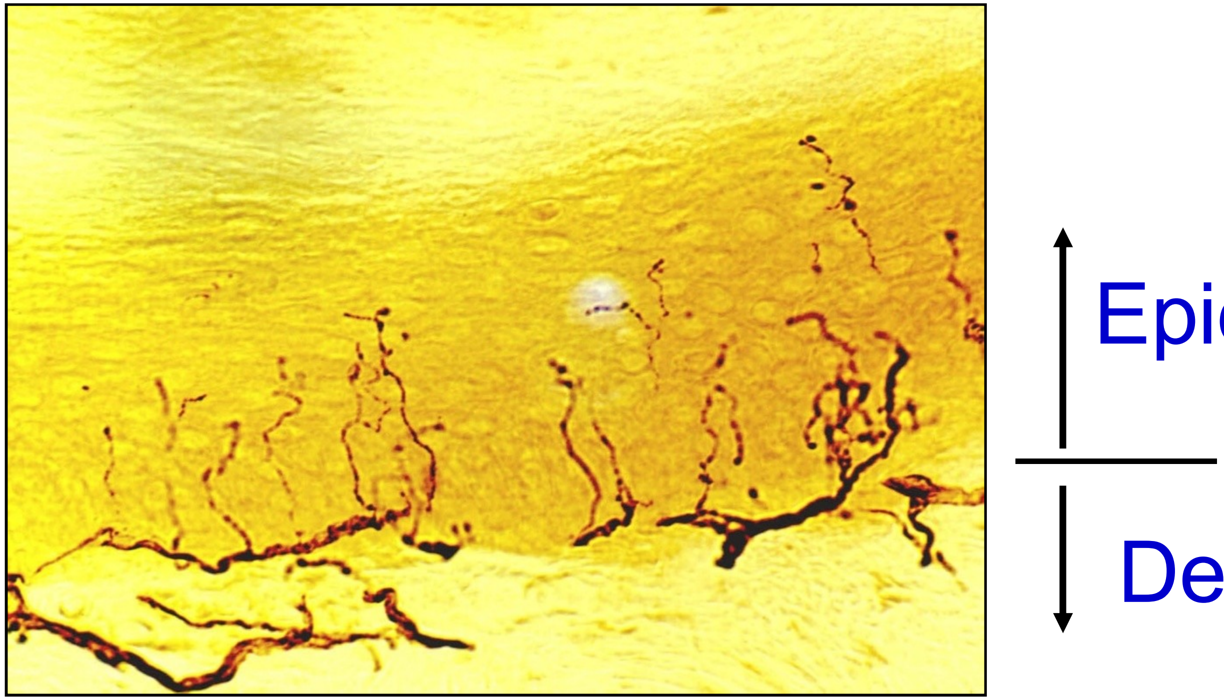
one year later



- 3 mm in diameter; no suture (only compression for several minutes is required)
- excellent wound healing within 2 weeks
- minimally invasive compared to standard sural nerve biopsy


epidermis
dermis
sg

Normal skin innervation



- Intrapeidermal nerve fiber (IENF): varicose appearance in the epidermis; stained with anti-protein gene product 9.5 (PGP 9.5), a pan-axonal marker
- Quantifiable: IENF density

Punch skin biopsy for small fiber neuropathy

- Neuropathologic evidence of nerve degeneration especially at cutaneous nerve terminals
- Objective and quantifiable: intraepidermal nerve fiber density (IENF density) as skin innervation index parameter of cutaneous nerve terminal degeneration
- An established procedure for small fiber neuropathy: consensus of EU Neurological Societies Task Force

Successful regeneration in Linezolid (Zyvox)-induced neuropathy

Painful neuropathy with skin denervation after prolonged use of linezolid

C-C Chao,[1] H-Y Sun,[2] Y-C Chang,[1] S-T Hsieh[1,3]

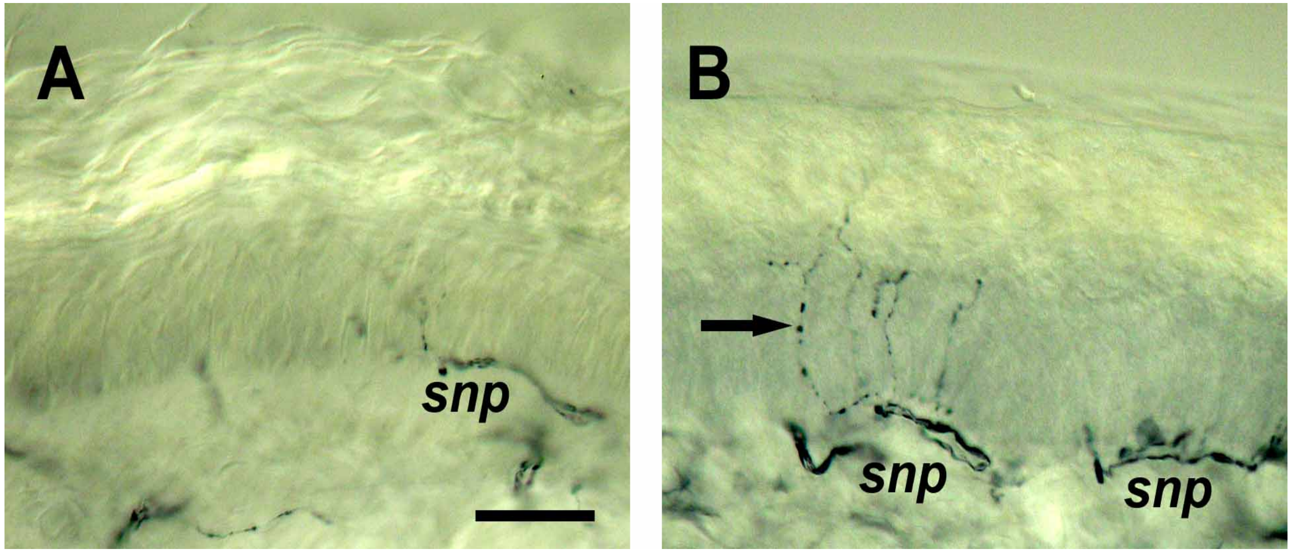
ABSTRACT

The prolonged use of linezolid, a new antibiotic against drug-resistant Gram-positive pathogens, might cause painful neuropathy. This finding raises the possibility that small-diameter sensory nerves in the skin, which are responsible for transmitting nociceptive information, might be affected. We report a 53-year-old female who developed pure small-fibre painful neuropathy (visual analogue scale, VAS = 82 on 0–100 scale) with marked skin denervation in the leg (epidermal nerve density, END = 2.32 fibres/mm, norm <5.88 fibres/mm) and significant elevation of the warm threshold in the foot (40.0°C, norm <39.4°C) after the use of linezolid for 6 months. Eight months after the discontinuation of linezolid, the skin became fully reinnervated (END = 9.04 fibres/mm), with disappearance of neuropathic pain (VAS = 0) and normalisation of the warm threshold (36.3°C). Nerve conduction studies for large-diameter motor and sensory nerves were normal. This report documents a pure small-fibre sensory neuropathy after prolonged use of linezolid, and the relationship between skin innervation and corresponding neuropathic pain.

In contrast to previous reports o
neuropathy associated with linezol
report a patient with pure small-
neuropathy and demonstrate the p
cutaneous nerve degeneration after lo
of linezolid and skin re-innervatio
cessation of the medication.

Zyvox use of 6 months:
- VAS=82; END=2.32
- Warm threshold=40.0 ^{o}C

8 months after DC of Zyvox:
- VAS=0; END=9.04
- Warm threshold=36.3 ^{o}C



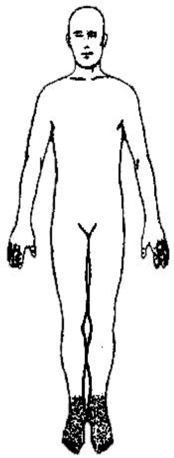
(Chao et al, JNNP 2008)

Skin biopsy in Diabetic neuropathy

DOI: 10.1093/brain/awh180 *Brain* (2004), **127**, 1593–1605

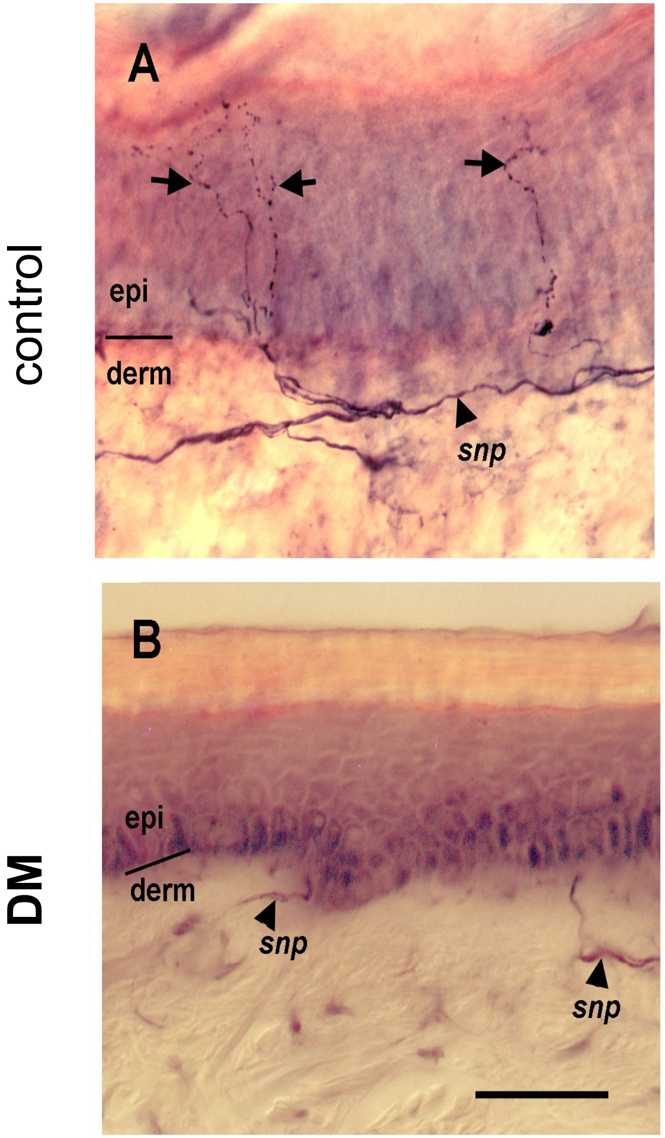
Skin denervation in type 2 diabetes: correlations with diabetic duration and functional impairments

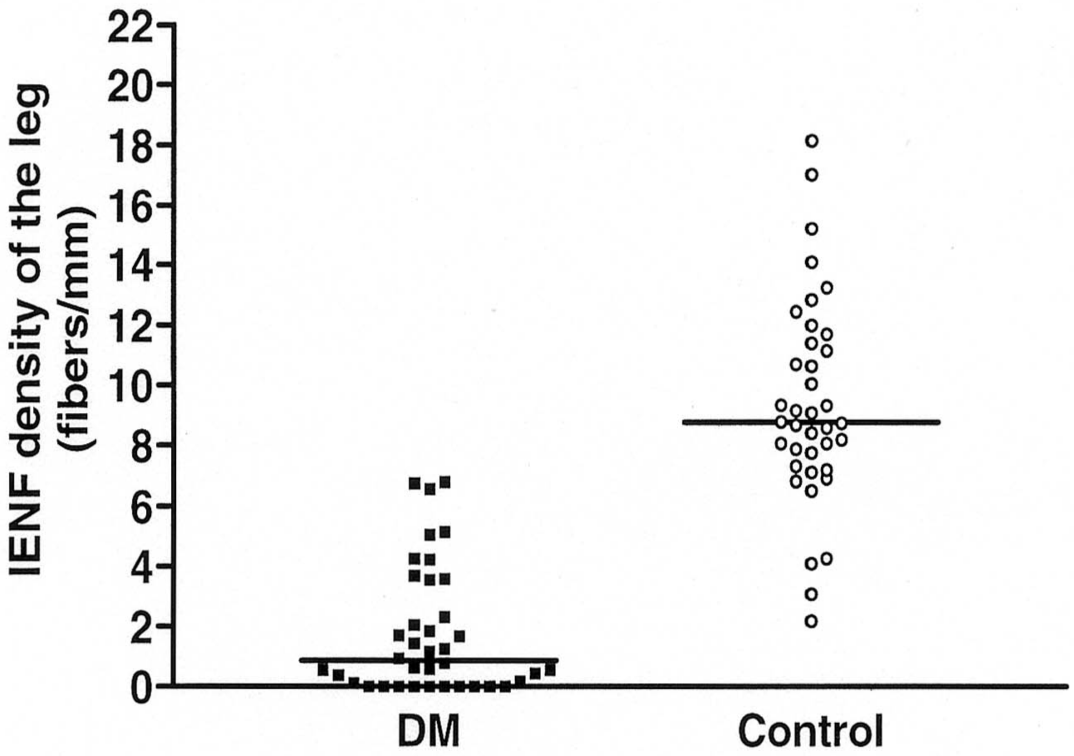
Chia-Tung Shun,[1,4] Yang-Chyuan Chang,[2] Huey-Peir Wu,[3] Song-Chou Hsieh,[3] Whei-Min Lin,[5] Yea-Hui Lin,[2] Tong-Yuan Tai[3] and Sung-Tsang Hsieh[2,5]



- Diabetic patients with length-dependent symptoms
- Skin biopsy and quantitative sensory test (warm threshold and cold threshold)

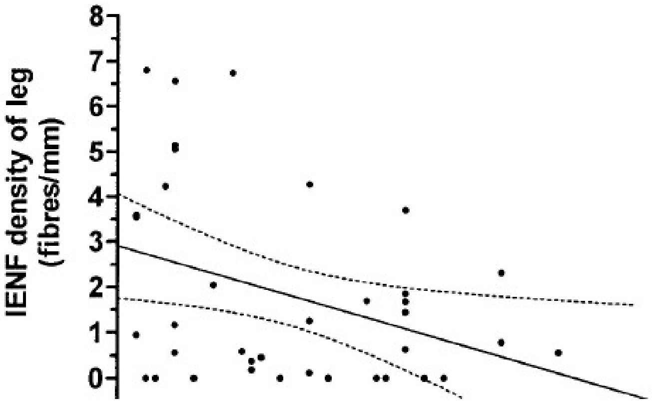
Skin nerve degeneration in diabetes:
reduced intraepidermal nerve fiber (IENF) density

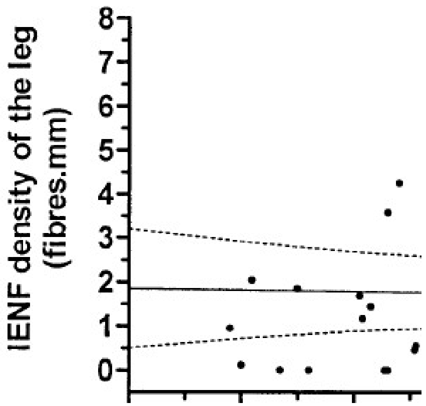


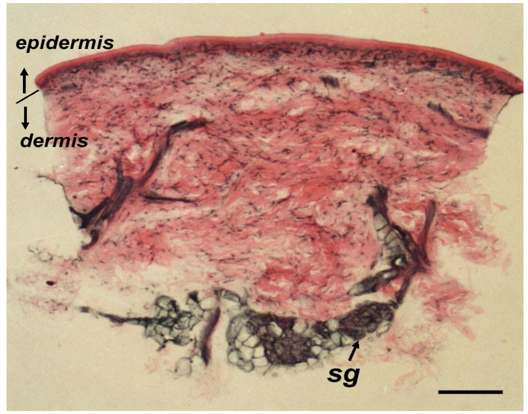


(Shun et al, Brain, 2004)

impaired nerve regeneration in diabetes



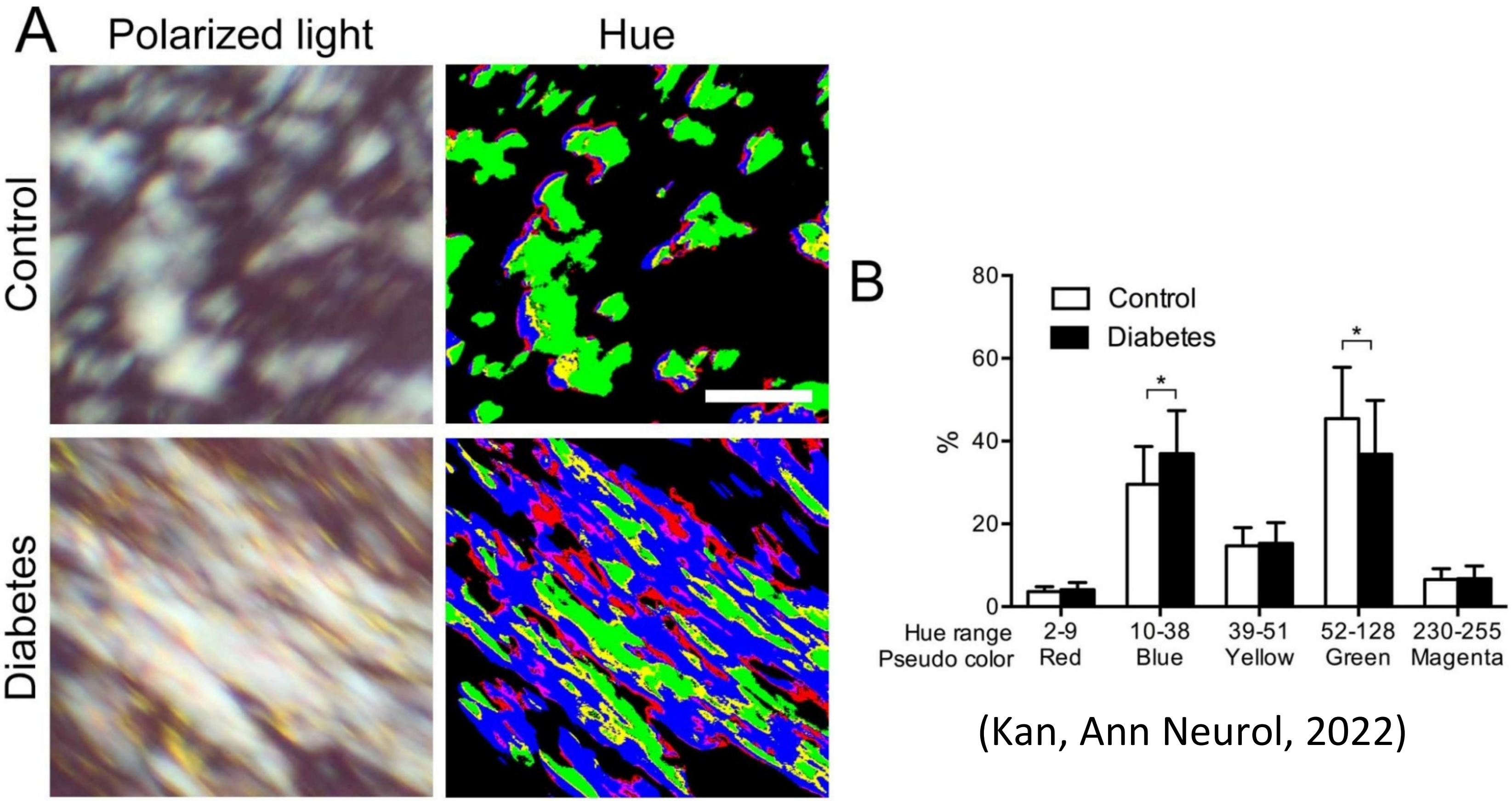




- Skin denervation persisted despite the improvement of glycemic control (Shun, 2004)
- Experimental approaches
  - Human tissues: Diabetic skin
  - Cell model: Dorsal root ganglia (DRG) culture
    - Co-culture with players in the microenvironment: fibroblast, endothelial cells, Schwann cells etc

Collagen contents in diabetic skin

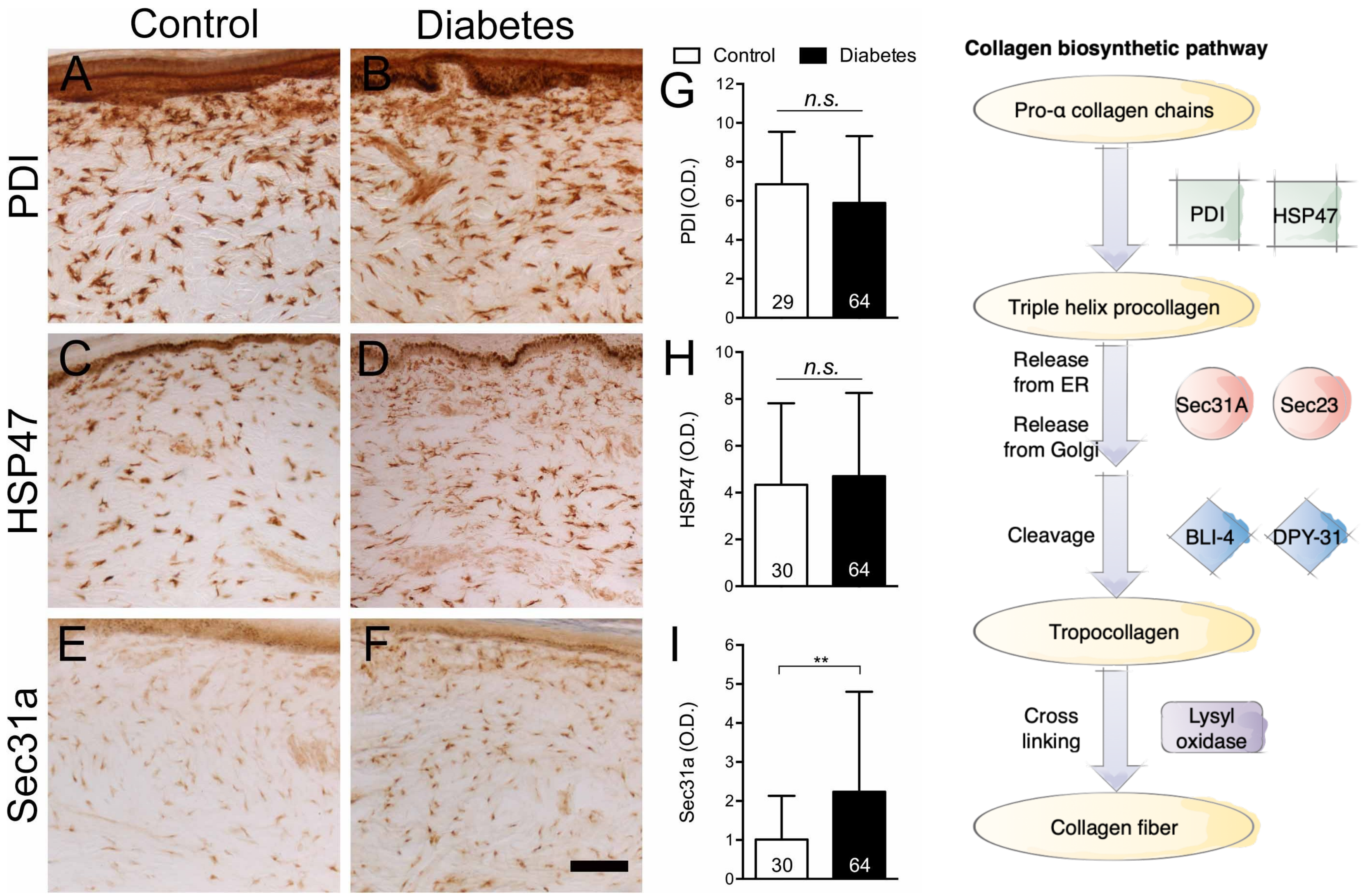
- Picrosirus staining for collagen contents
- Under polarized microscopy: Alteration of collagen contents in diabetic skin: aligned and tight



(Kan, Ann Neurol, 2022)

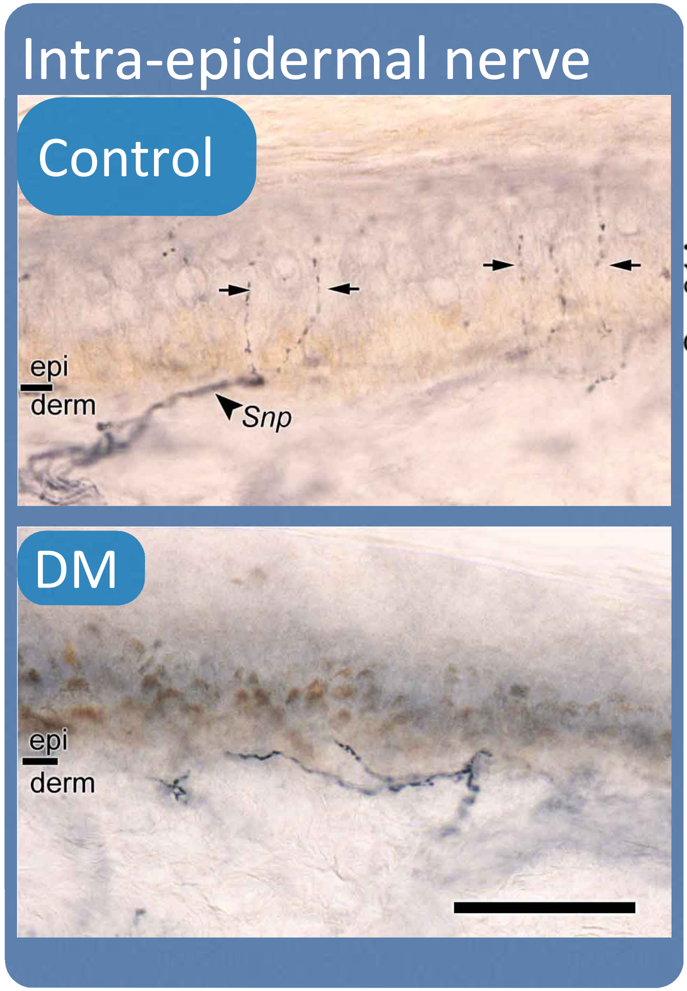
Collagen biosynthesis in diabetes

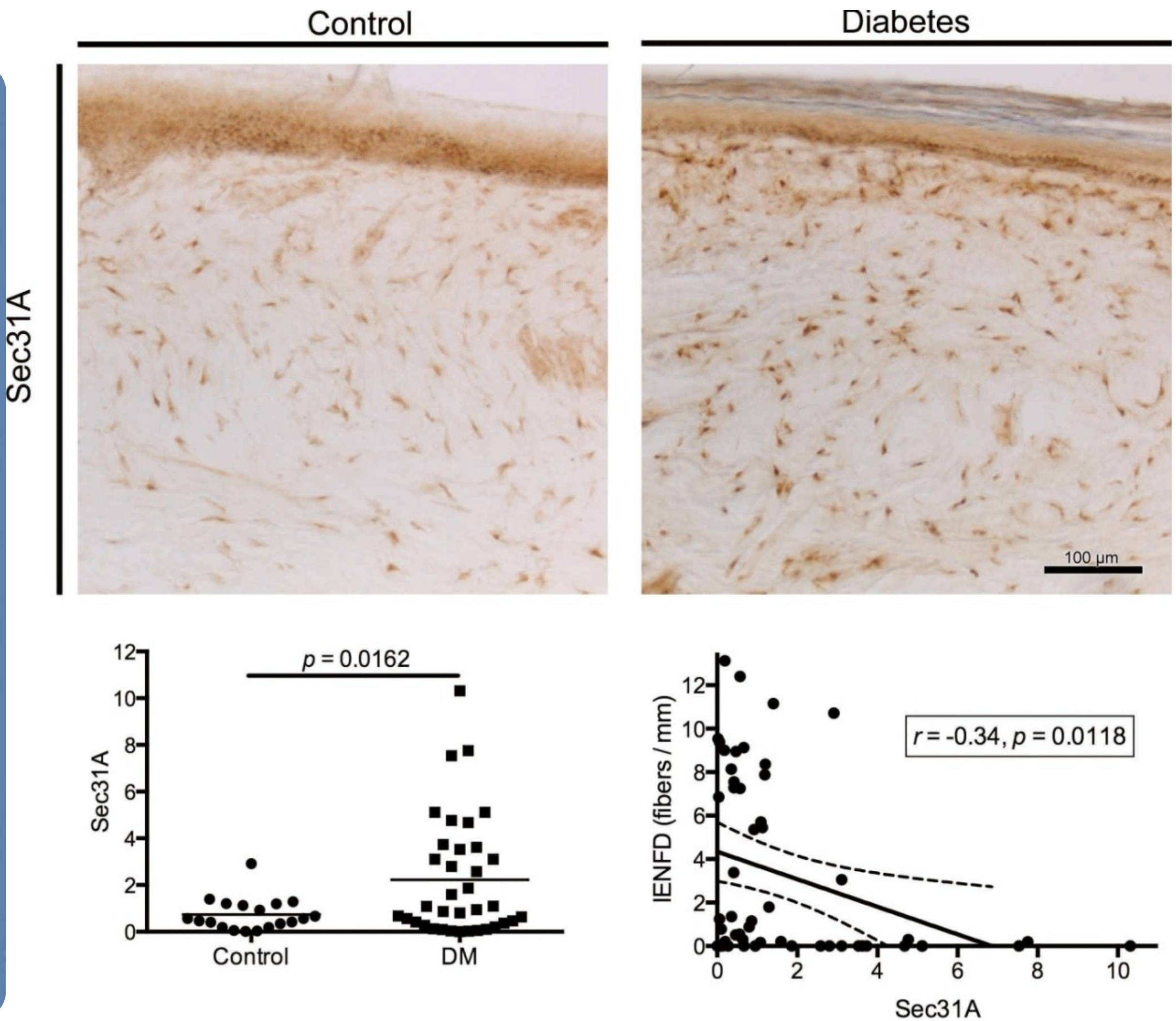
- Increased Sec31a in diabetic skin



(Kan, Ann Neurol, 2022)

Sec31a upregulation in the DM skin and correlated with IENFD

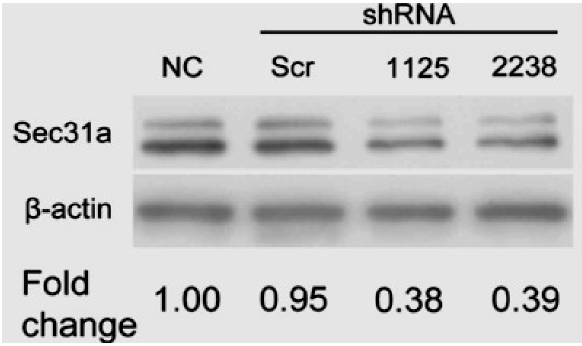


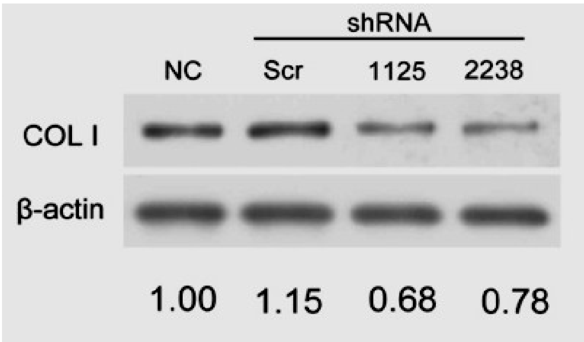


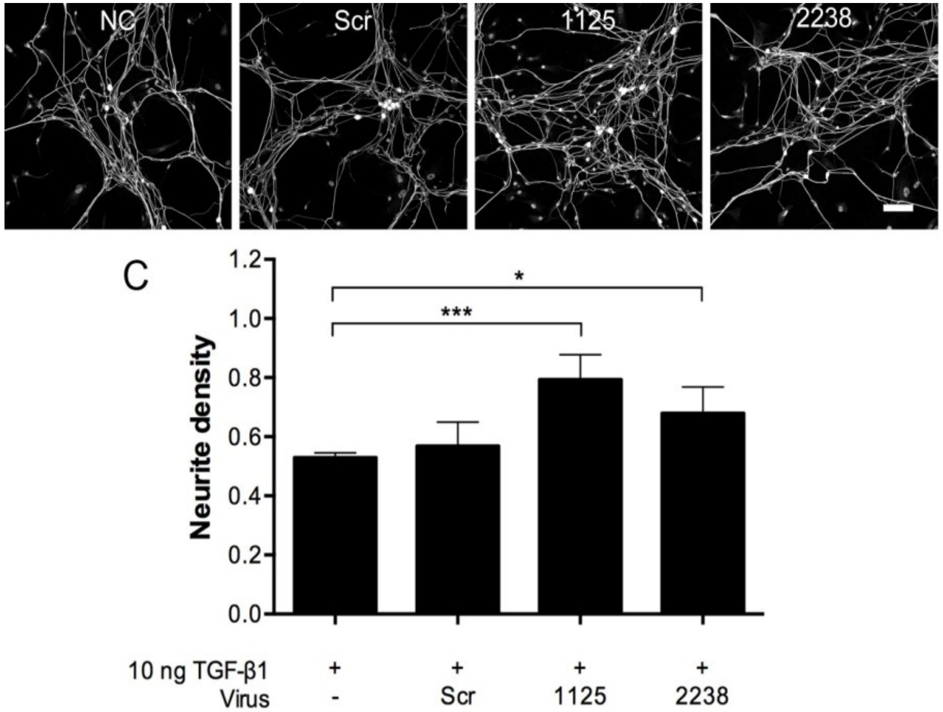
(Kan, Ann Neurol, 2022)

Silencing Sec31a

- downregulated collagen I expression
- reversed on neurite outgrowth



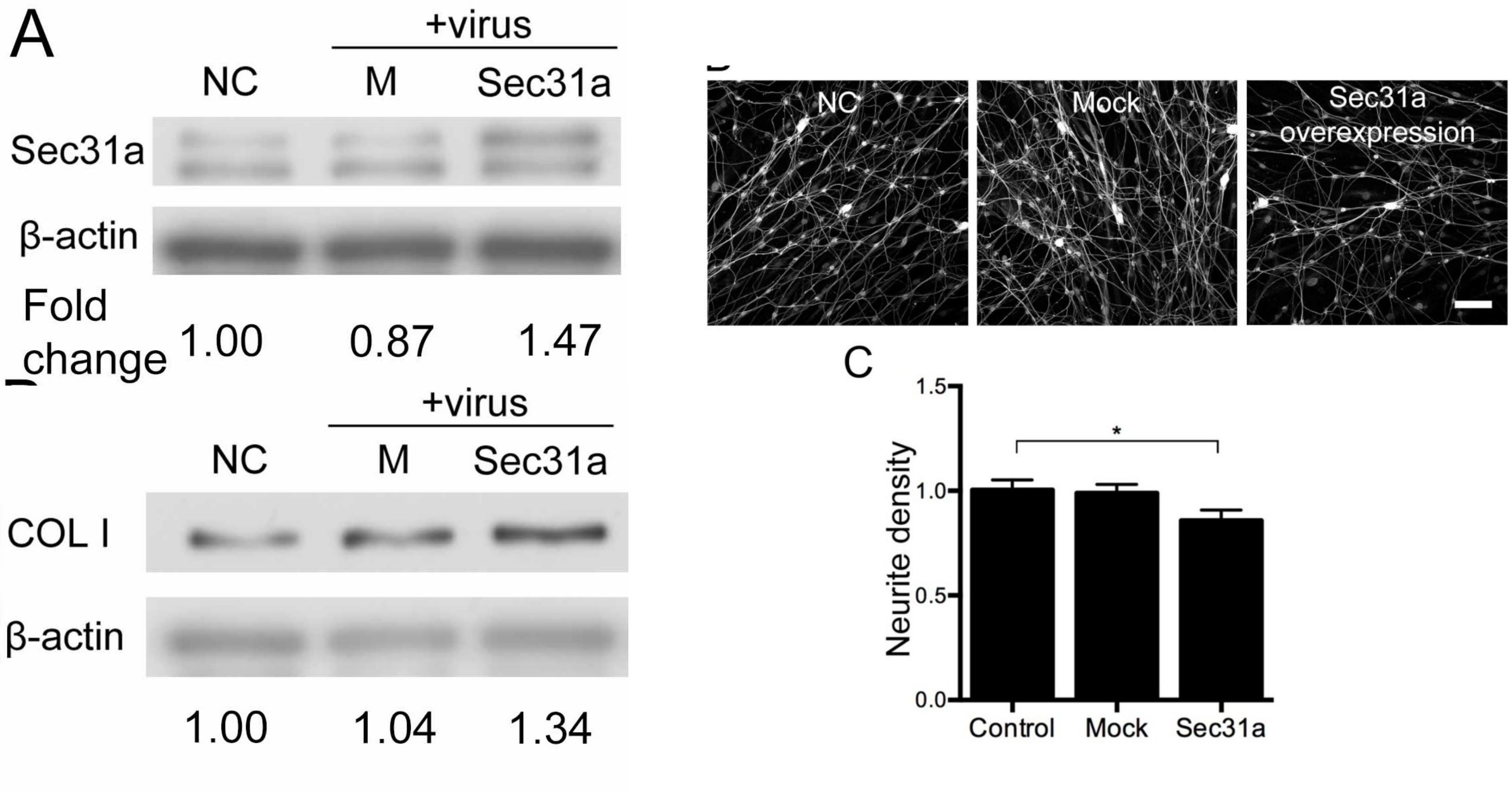




(Kan, Ann Neurol, 2022)

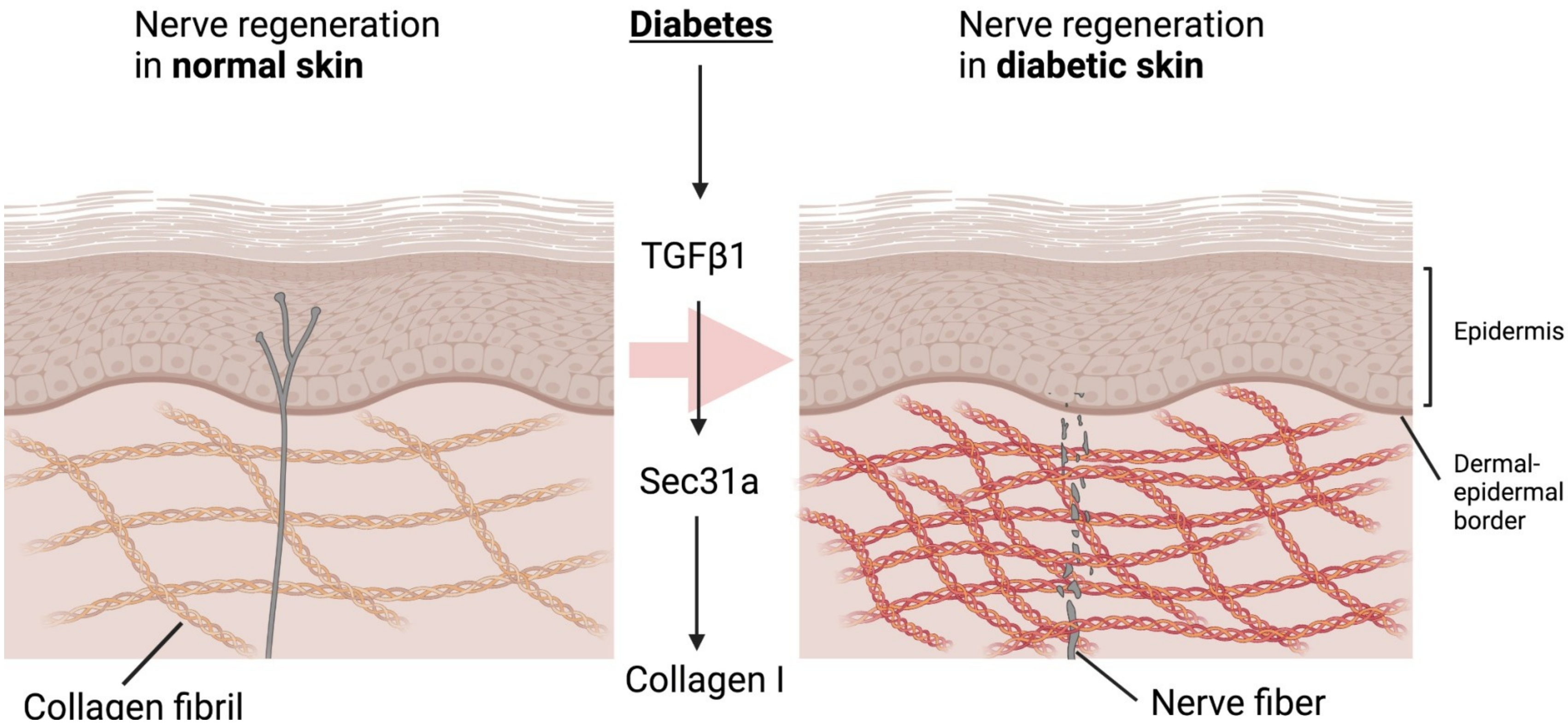
Over-expression of Sec31a

- upregulated collagen I expression
- Impaired neurite outgrowth



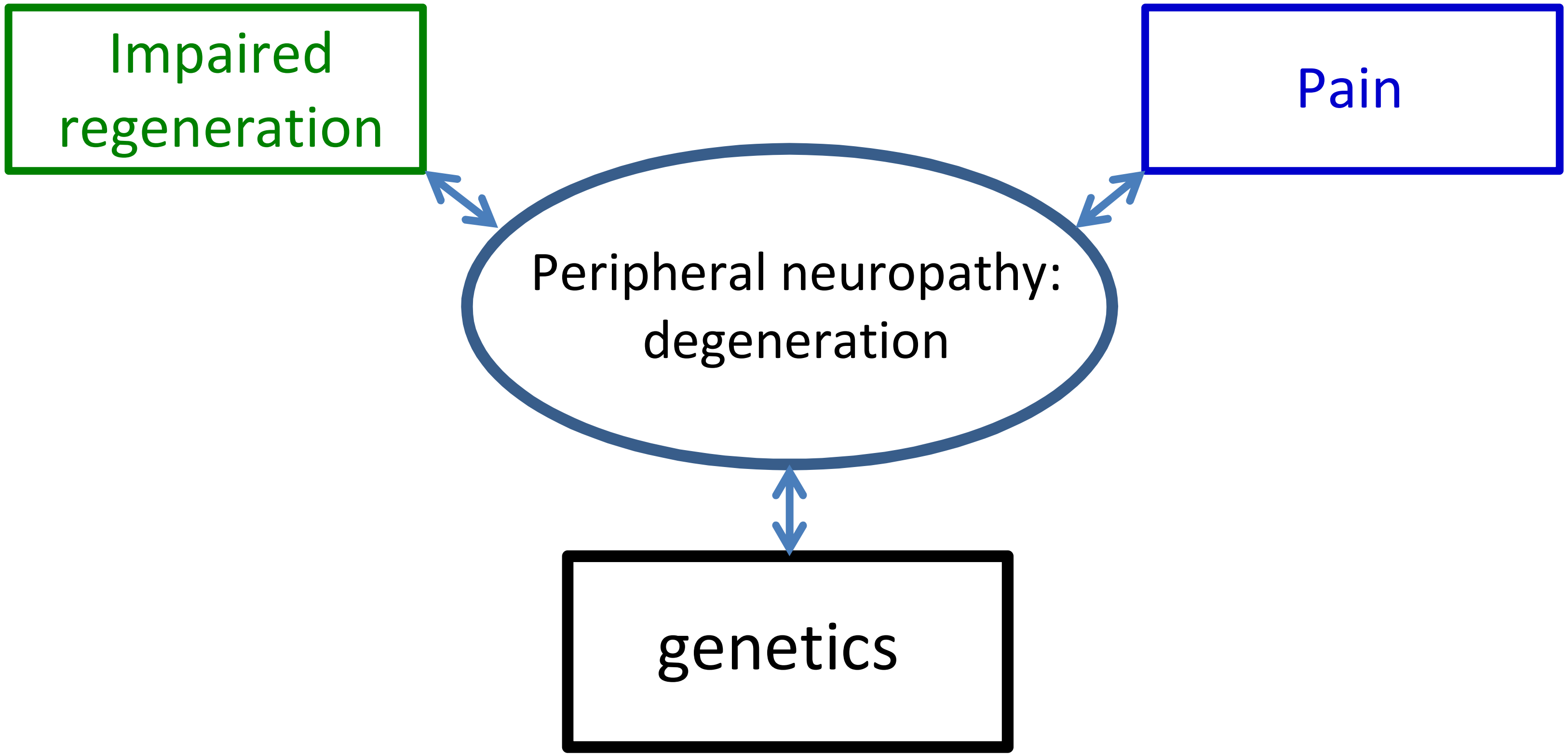
(Kan, Ann Neurol, 2022)

Sec31a impaired nerve regeneration in diabetes



(Kan et al, Ann Neurol, 2022)

Small fiber neuropathy: degeneration and pain

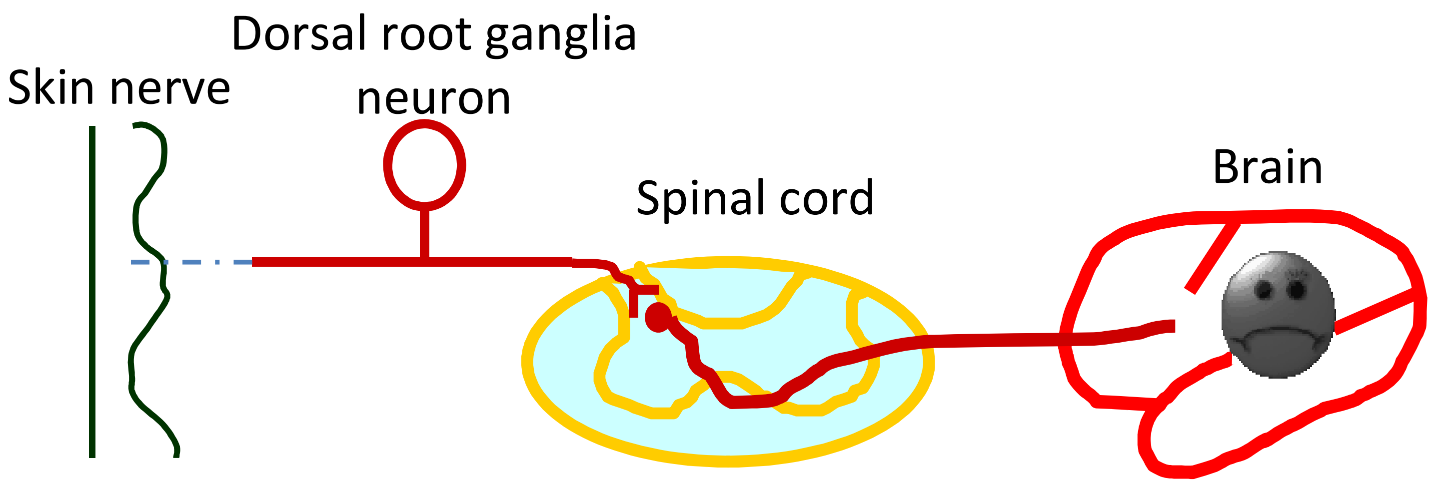


nerve degeneration and pain in small fiber neuropathy: peripheral sensitization?

Table 7 *Relationship between intraepidermal nerve fibre density and amplitude of sural sensory ac clinical parameters*

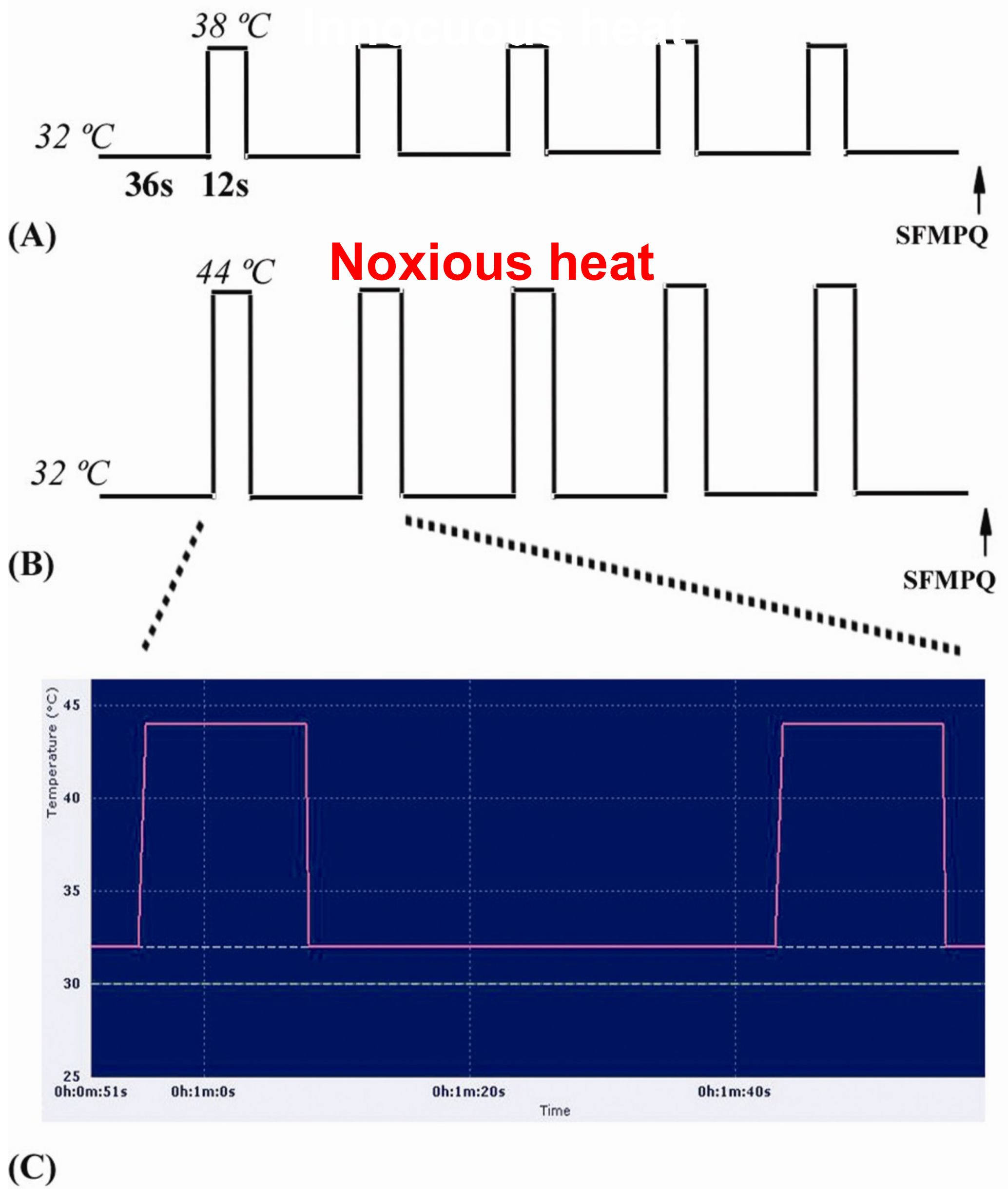
		IENF density (fibres/mm)		Sural SAP
		Median (range)	*P*	Median (ra
Painful neuropathy	Yes (9)†	0.17 (0–4.23)	0.1137	4.2 (0–13.1
	No (29)	1.44 (0–6.80)		8.95 (0–18.
Symptomatic site	Yes (7)	0 (0–1.68)	0.0116*	0 (0–4.96)
	No (31)	1.25 (0–6.8)		8.95 (0–18

Peripheral sensitization   Central sensitization



(Shun CT, Brain 127:1593-1605, 2004)

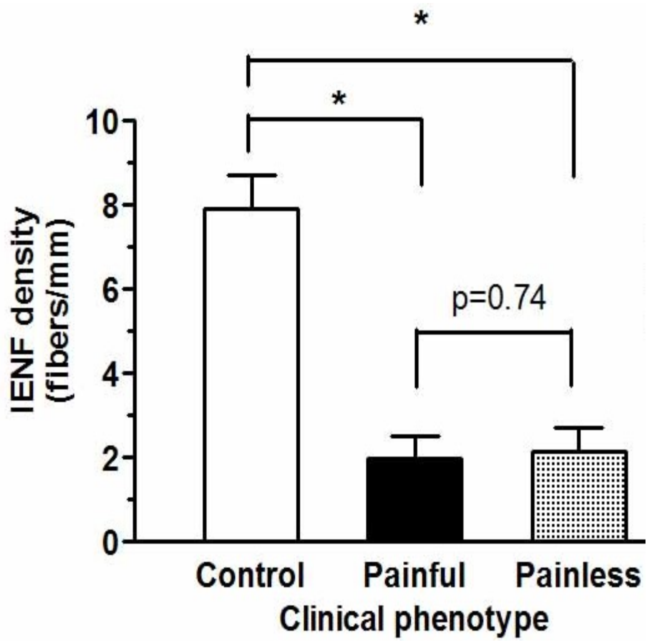
fMRI: imaging signatures of neuropathic pain



Tseng MT, Human Brain Mapping, 2013

Skin denervation in diabetes: clinical profiles

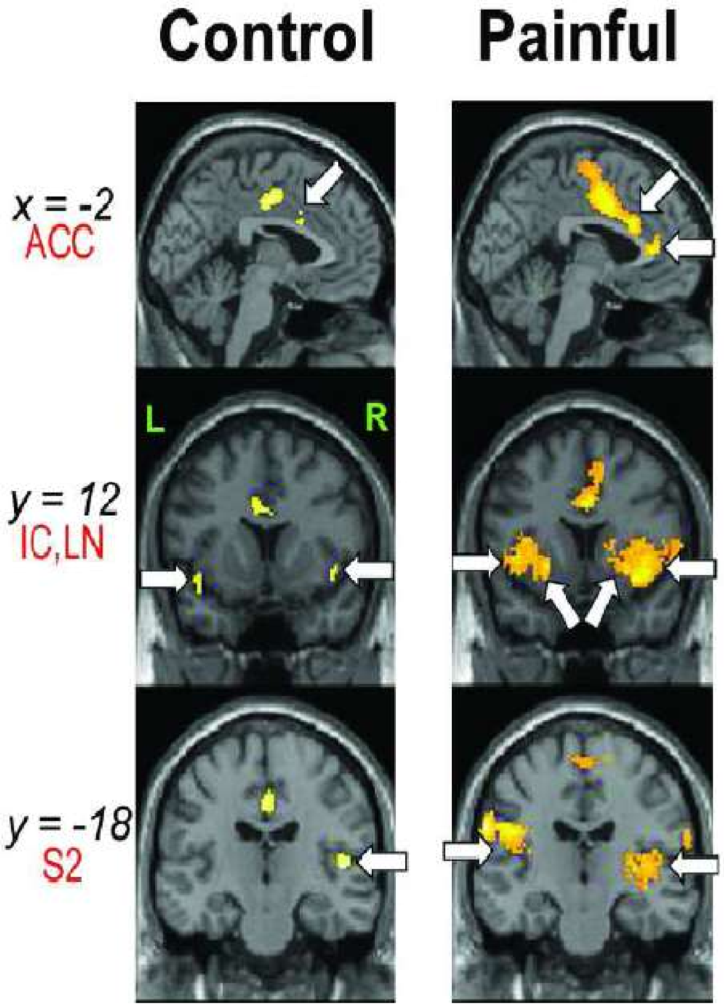
- type 2 diabetic patients: grouped by painful (burning) vs. painless (numbness) with comparable degree of skin nerve (p = 0.74), but lower than controls (both $p < 0.0001$)
- comparable in age, gender, diabetic and glycemic status

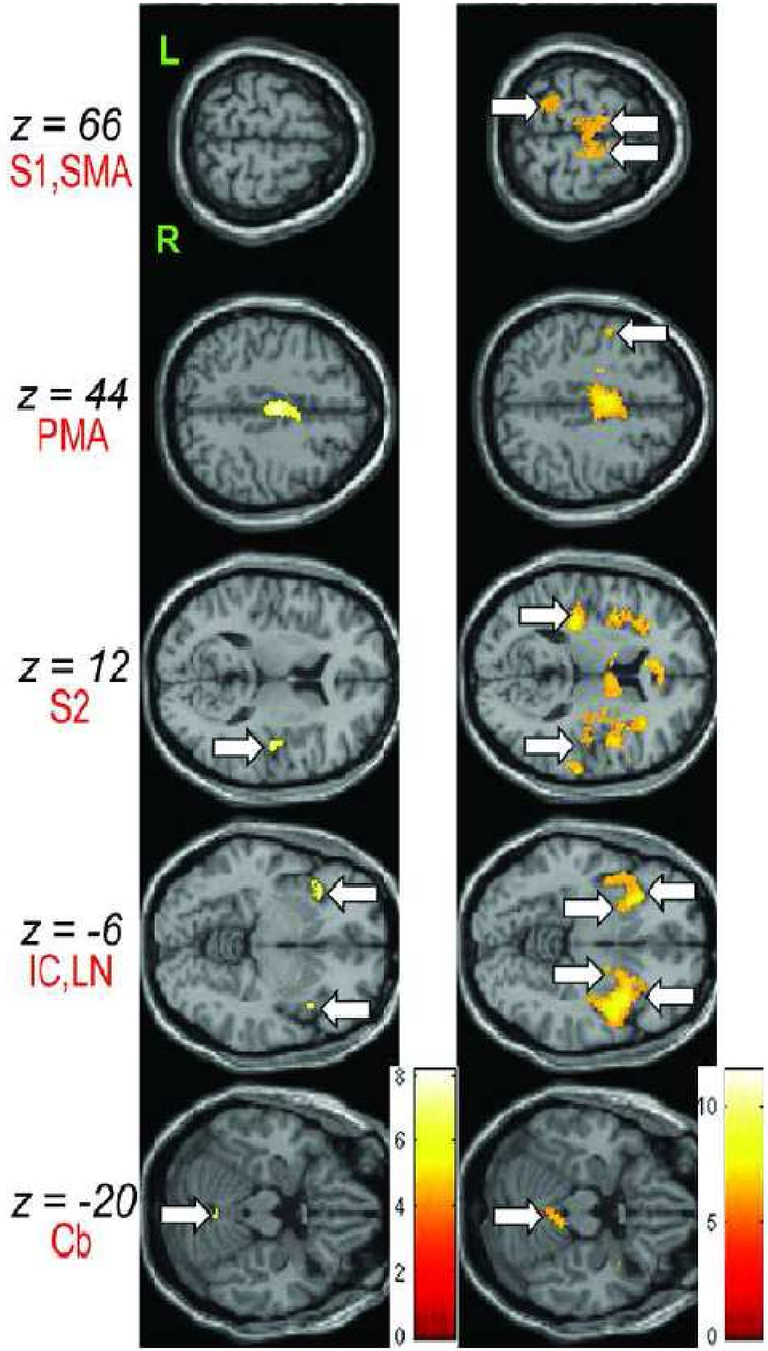


	Painful	Painless
Age (years) (range)	51.1 ± 9.1 (32–64)	51.3 ± 10.6 (25–65)
Gender (M/F)	4/7	6/5
Duration of diabetes (years)	6.0 ± 3.2	6.6 ± 6.2
NOD [median (range)]	1 (0–3)	1 (0–2)
Symptoms [*n*, median (range)]		
Spontaneous pain		
Burning	11, 5 (3–10)	0, 0 (0)
Electrical shocks/stabbing	5, 0 (0–5)	0, 0 (0)
Stimulus-evoked pain		
Thermal hyperalgesia	11, 6 (2–10)	0, 0 (0)
Mechanical allodynia	1, 0 (0–5)	0, 0 (0)
Paresthesias/dysesthesias		
Numbness	8, 5 (0–10)	11, 5 (3–10)

(Tseng et al, Human Brain Mapping, 2013)

Brain activations in diabetic neuropathy





Tseng et al, Human Brain Mapping, 2013

Enhanced brain activations in small fiber neuropathy of diabetes

(1) Somatosensory system: thalamus;

(2) Limbic system: Hippocampus;

(3) Lentiform nucleus (LN)

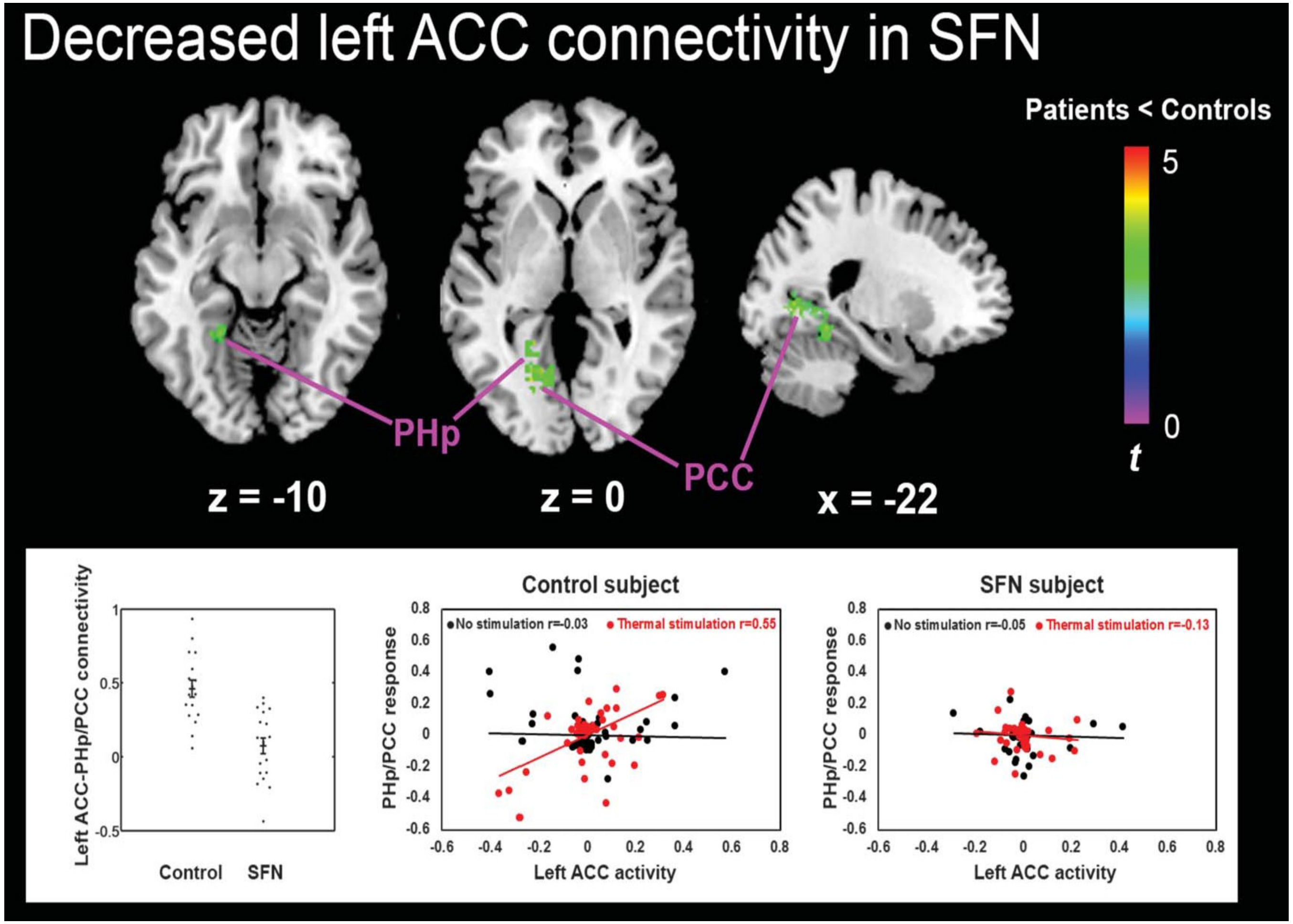
TABLE III. Group activation areas of analysis of covariance

Cluster size (voxels)	Region	Side	BA	*F* [MNI Coordinates: x, y, z (mm)]
614	ACC	L	24	14.34 (-6, 34, 8)
	SFG	L	9	12.24 (-12, 34, 36)
569	IC	R	-	8.53 (34, 16, -8)
143	Thalamus	L	-	6.5 (-6, -22, 0)
512	LN	R	-	10.41 (26, -16, 8)
	Hippocampus	R	-	9.61 (40, -24, -10)
74	PMA	L	6	8.62 (-20, -6, 50)
427	TG	L	22	8.97 (-46, -6, -6)
161	TG	R	22	8.58 (52, -50, 6)

Tseng et al, Human Brain Mapping, 2013

Decreased fcMRI in neuropathic pain

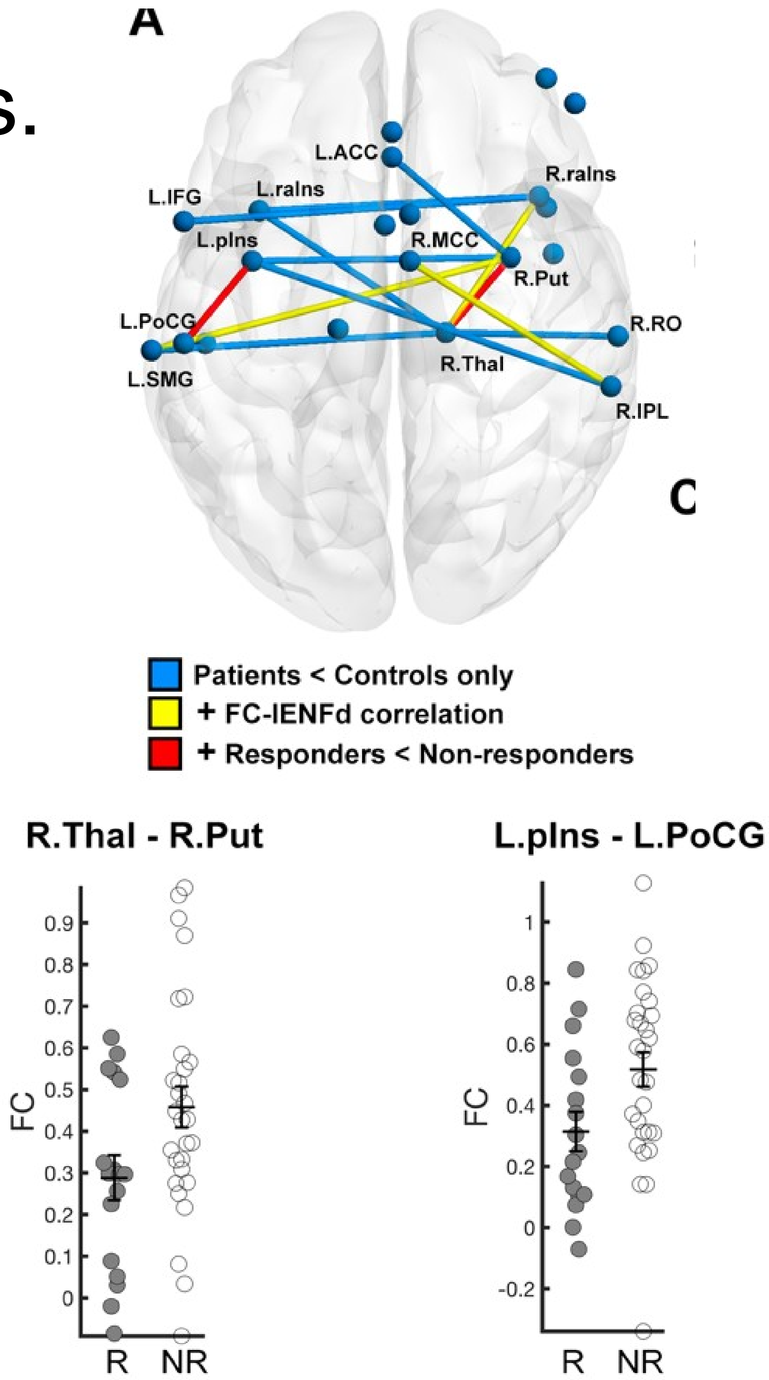
- reduced fc in small fiber neuropathy with reduced IENF density: ACC



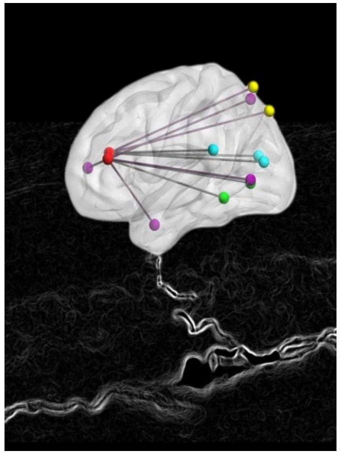
(Hsieh et al, Pain 156:904-916, 2015)

Functional connectome vs. treatment response

- Resting-state functional connectome: correlation to treatment response
- Responder had lower pre-treatment connectivity
  - (1) between the right thalamus and right putamen,
  - (2) between the left posterior insula and left postcentral gyrus (somatosensory cortex)



(Chao, Ann Neurol, 2023)

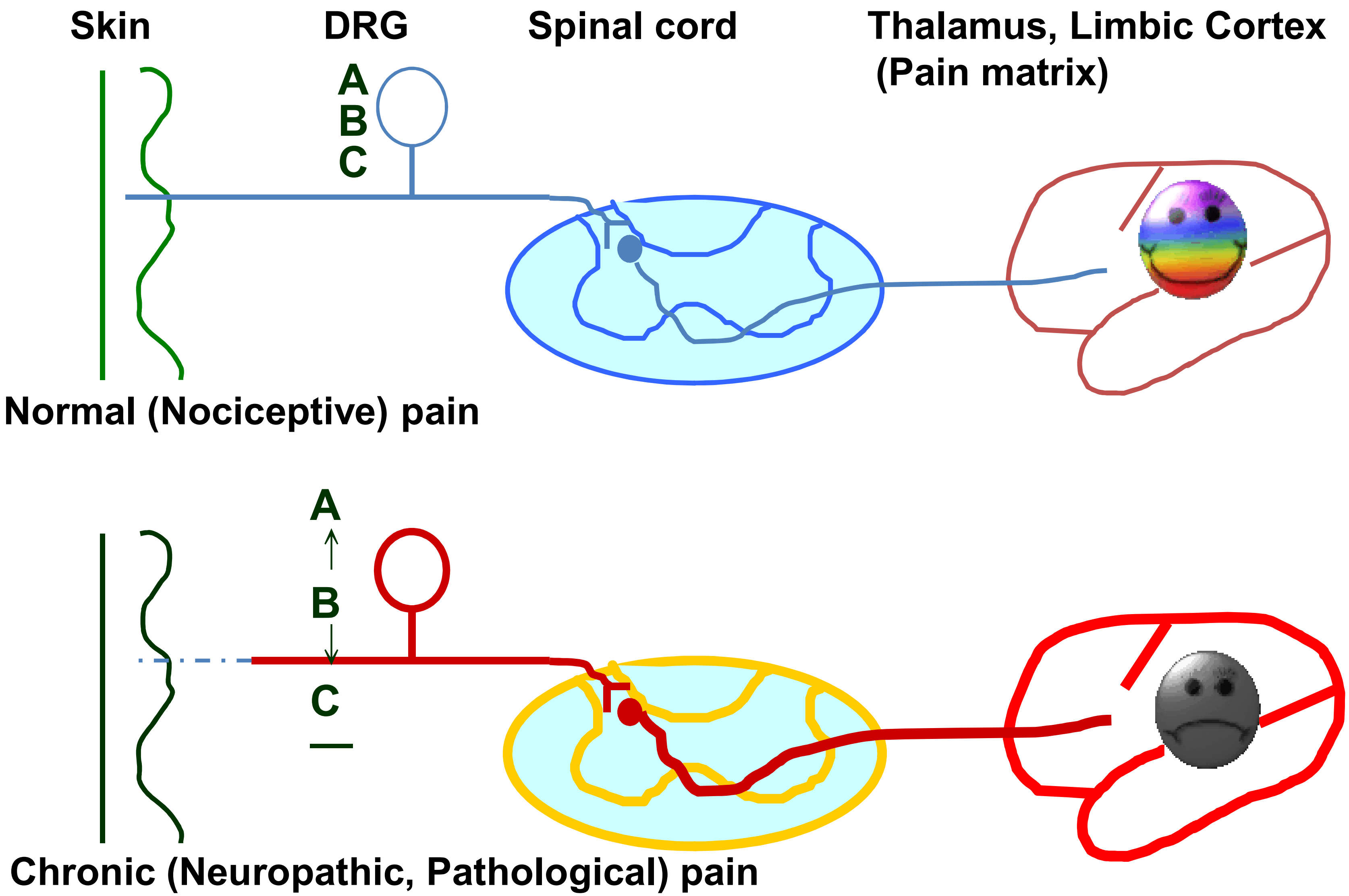


Altered connectome in neuropathic pain due to skin nerve degeneration in small fiber neuropathy

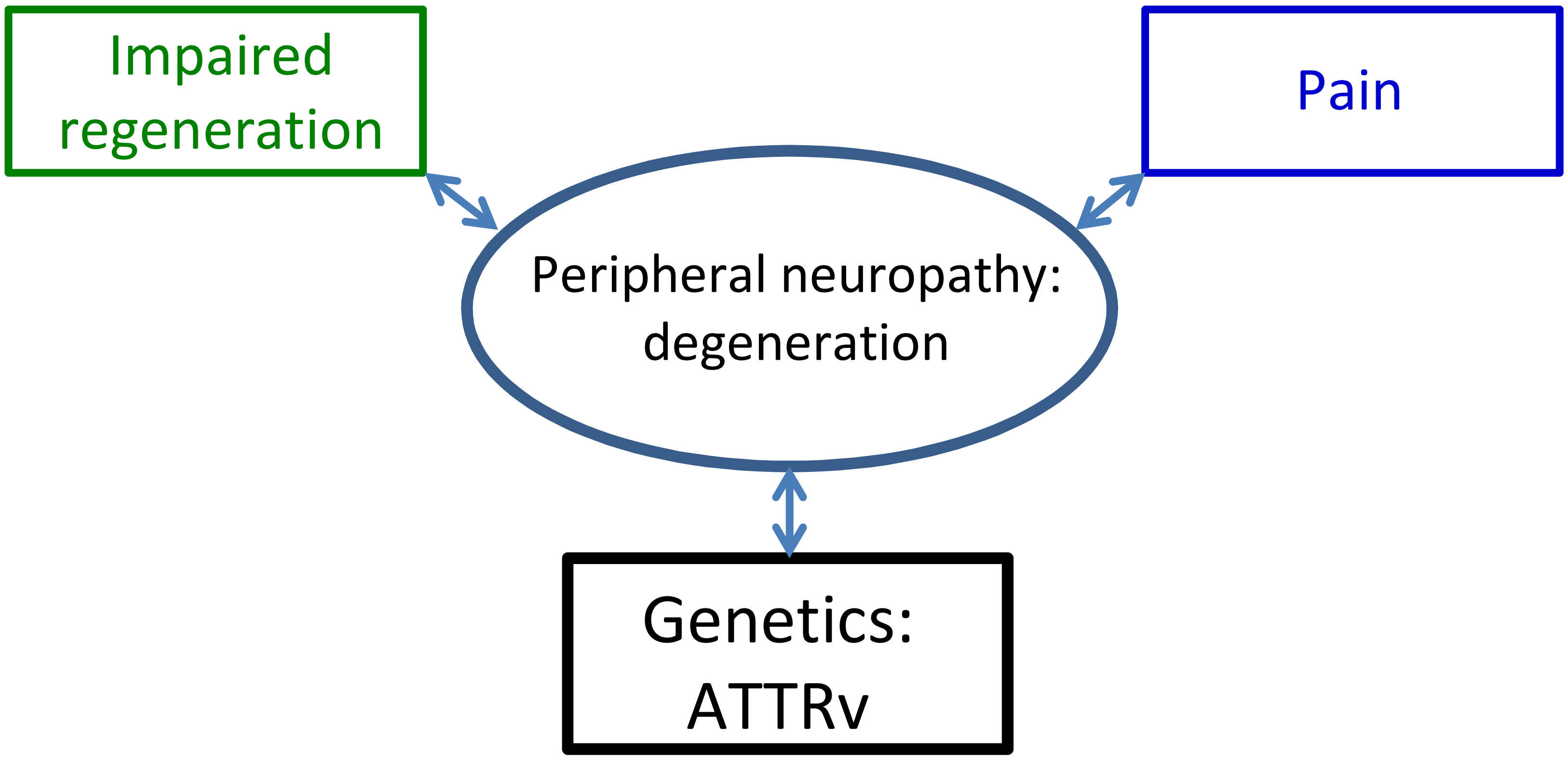
- no change in fractional anisotropy and gray matter volume in small fiber neuropathy
- reduced functional connectivity and structural connectome in neuropathic pain of small fiber neuropathy
- correlation of connectivity with IENF density
- the pain-related brain networks tend to break into functionally and structurally independent components, with severity linked to the degree of skin nerve degeneration in small fiber neuropathy

(Hsieh, Pain, 2015; Chao, Pain, 2021; Chao, Ann Neurol, 2023)

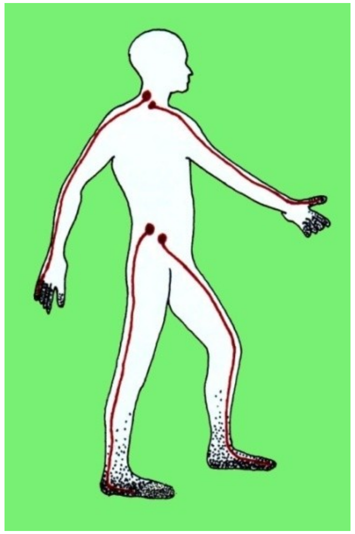
Brain plasticity in small fiber neuropathy



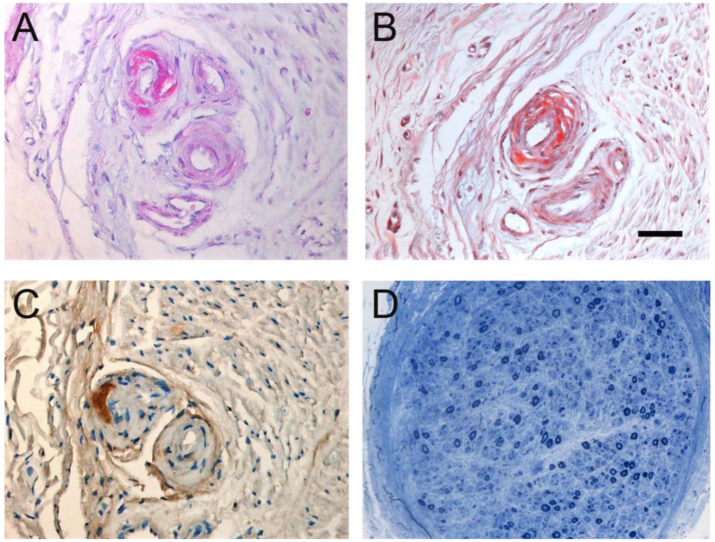
Small fiber neuropathy: degeneration and pain



“Idiopathic” axonal neuropathy in Taiwan

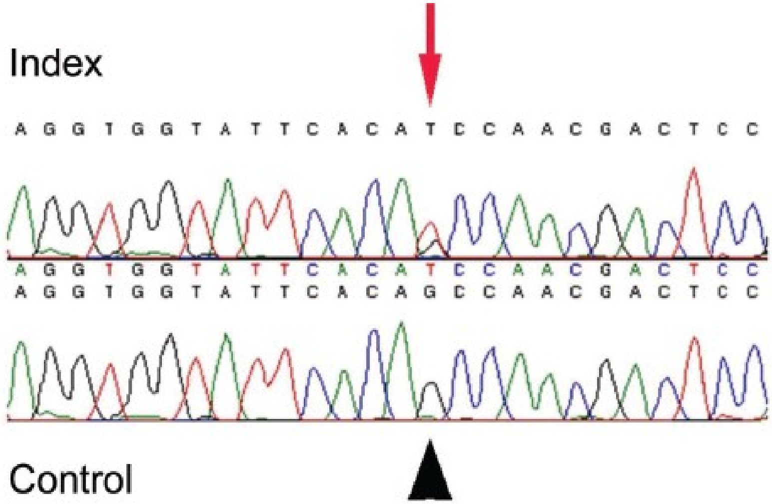


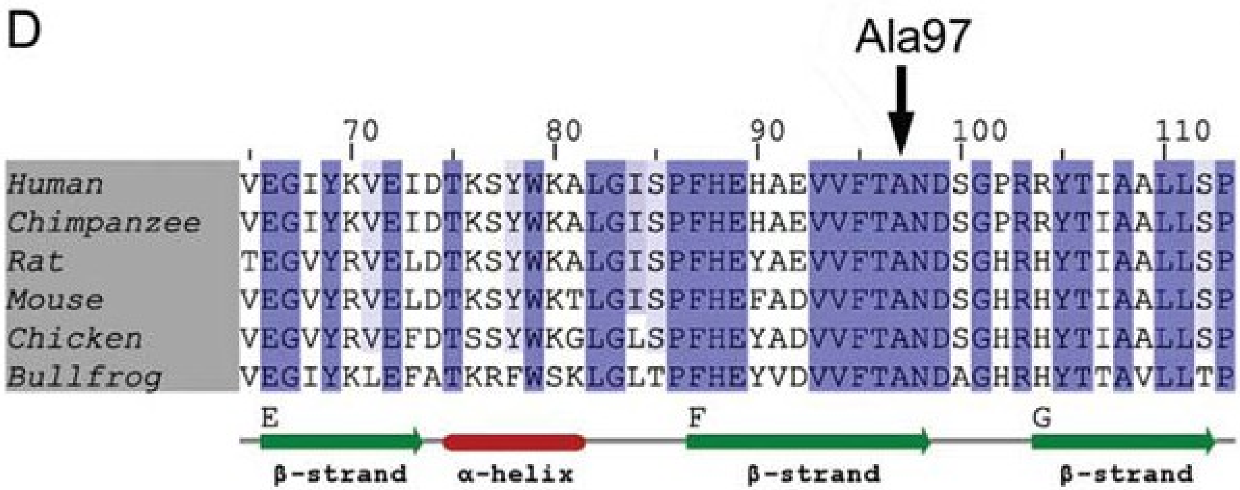
- A 54-year-old male started to have sensory symptoms of small fiber type. Unsteadiness, weakness, and orthostatic hypotension developed 3 years later.
- Nerve conduction studies: polyneuropathy of axonal degeneration
- Sural nerve: Congo-red for amyloid(+)

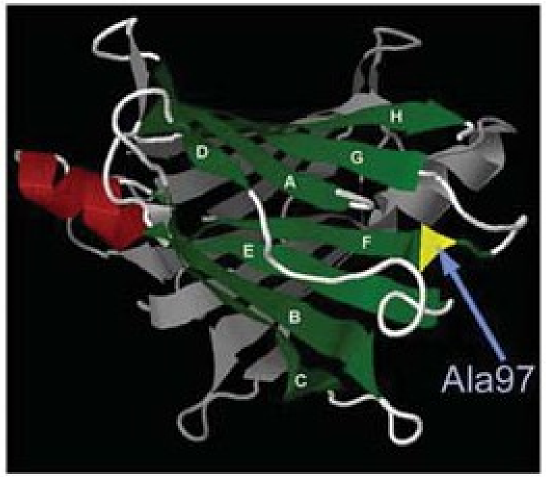


Hereditary transthyretin amyloidosis due to variant transthyretin TTR-A97S

- Mutation of G to T to cause transthyretin (Ala97Ser)
- Ala97: conserved
- Ala97Ser: unique in Taiwanese; most frequent (19/30 of the original screening)



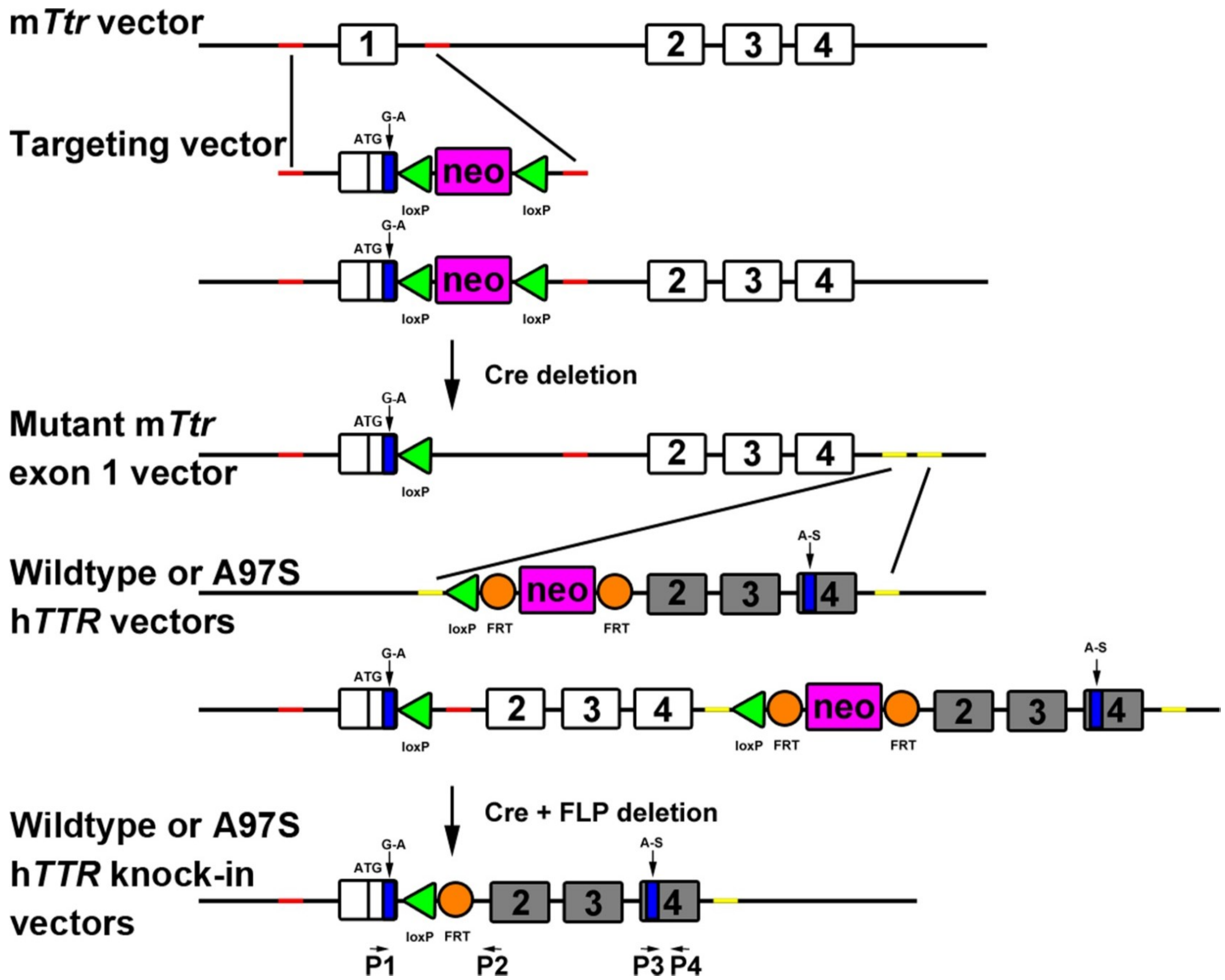




(Yang et al, Neurology 75:532–538, 2010)

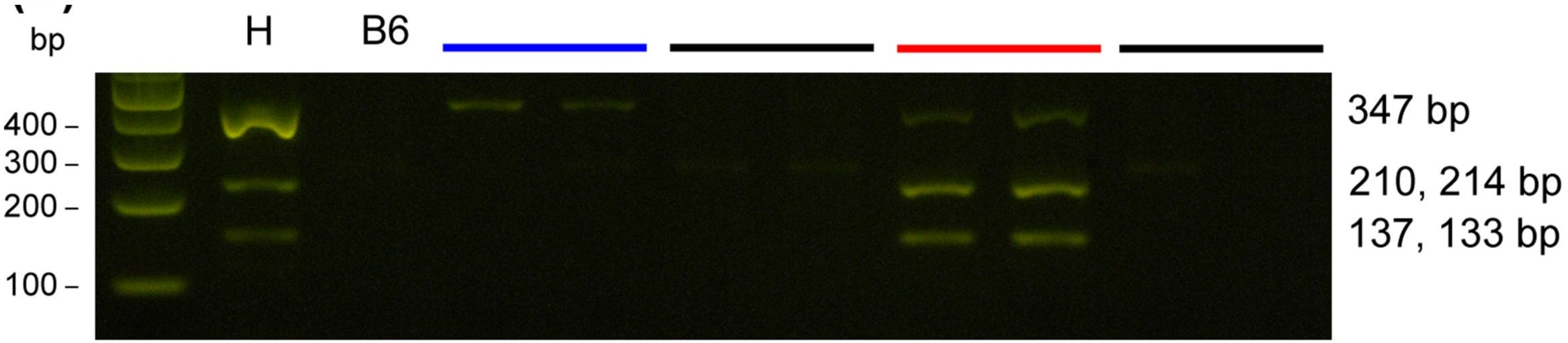
TTR-A97S knock-in mice

- generation of **wild type mice** ($hTTR^{wt}/hTTR^{wt}$) and **TTR-A97S mice** ($hTTR^{A97S}/hTTR^{wt}$)



Genetic confirmation of TTR-A97S

- absence of mouse TTR (mTTR) gene in knock-in mice
- presence of human TTR (hTTR) A97S gene in knock-in mouse



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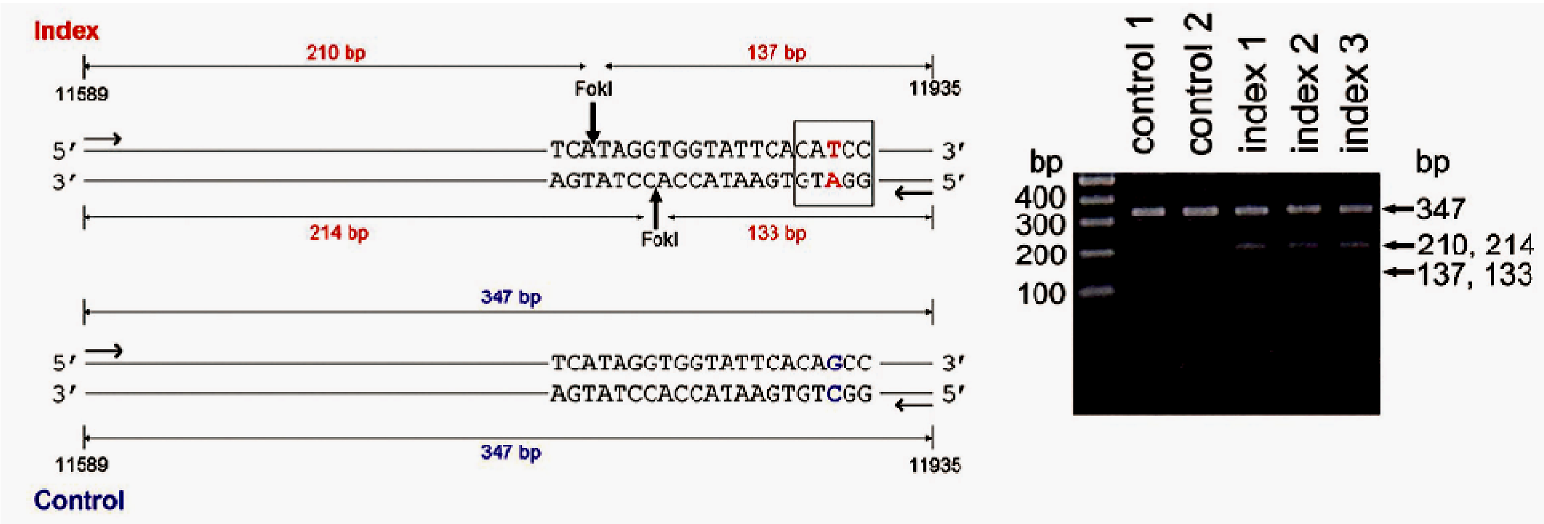
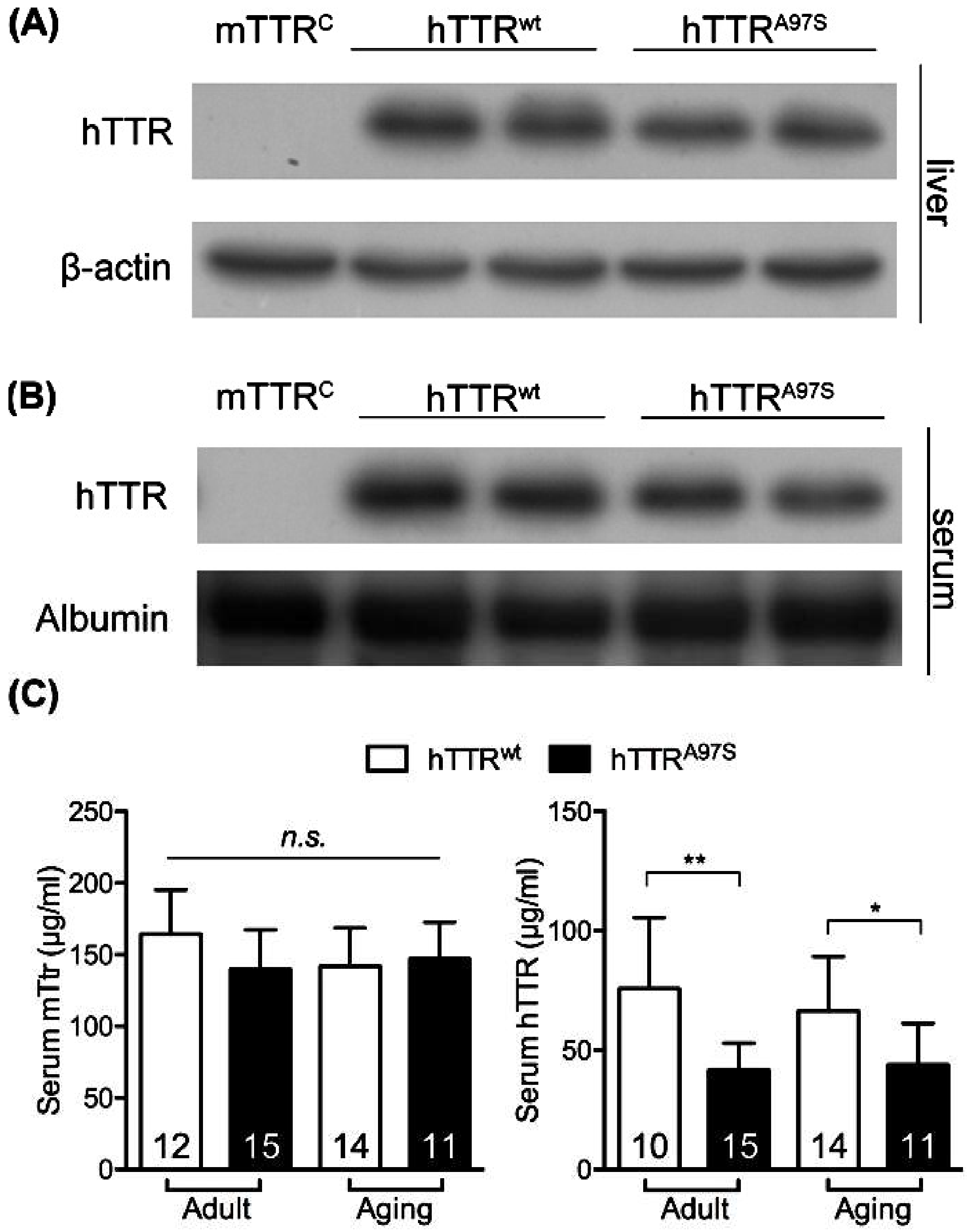


- mTTR^C
- hTTR^wt
- hTTR^A97S

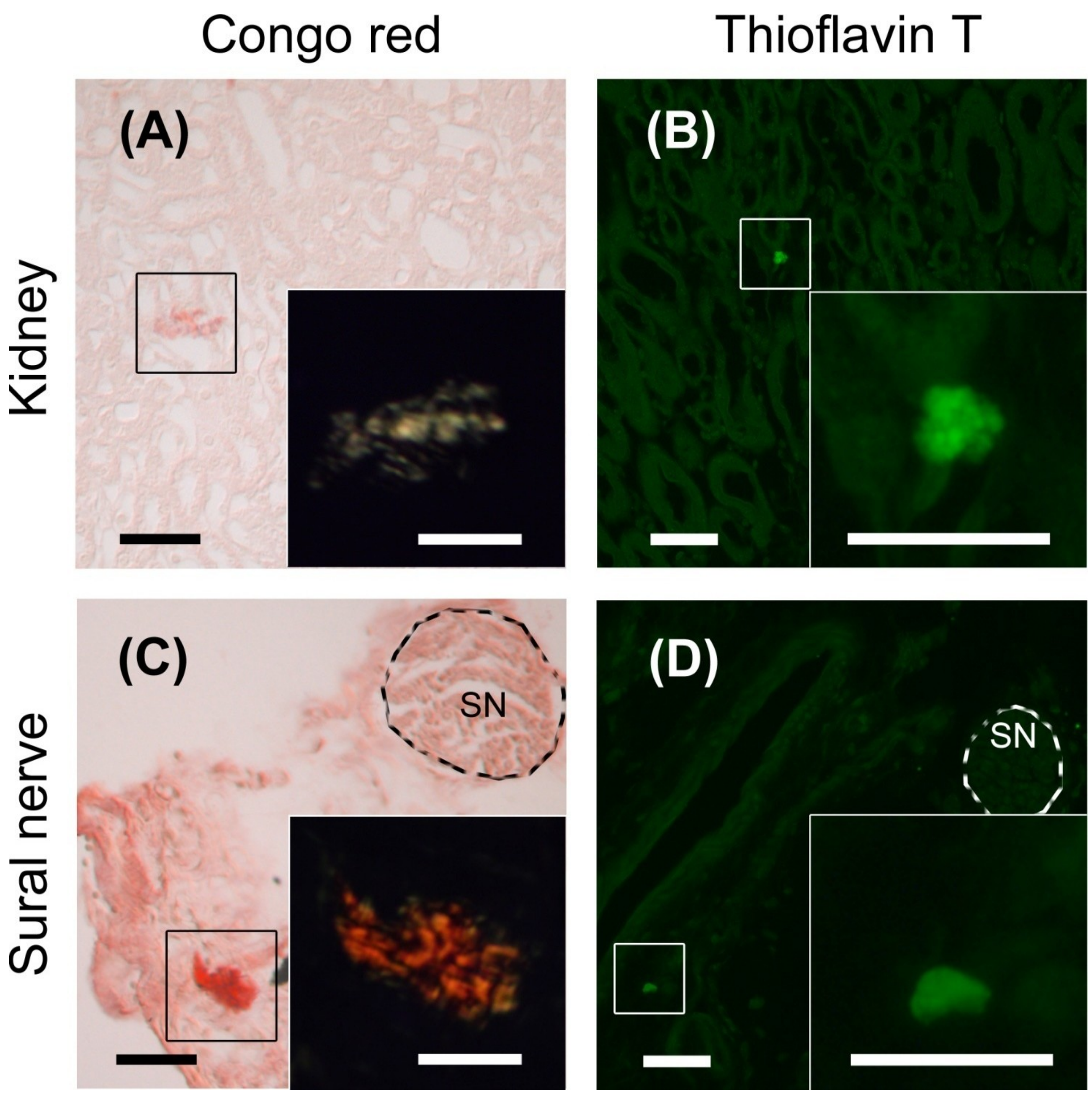
TTR protein expression

- presence of human TTR protein in liver and serum of knock-in mice
- ELISA: reduced serum TTR in knock-in mice



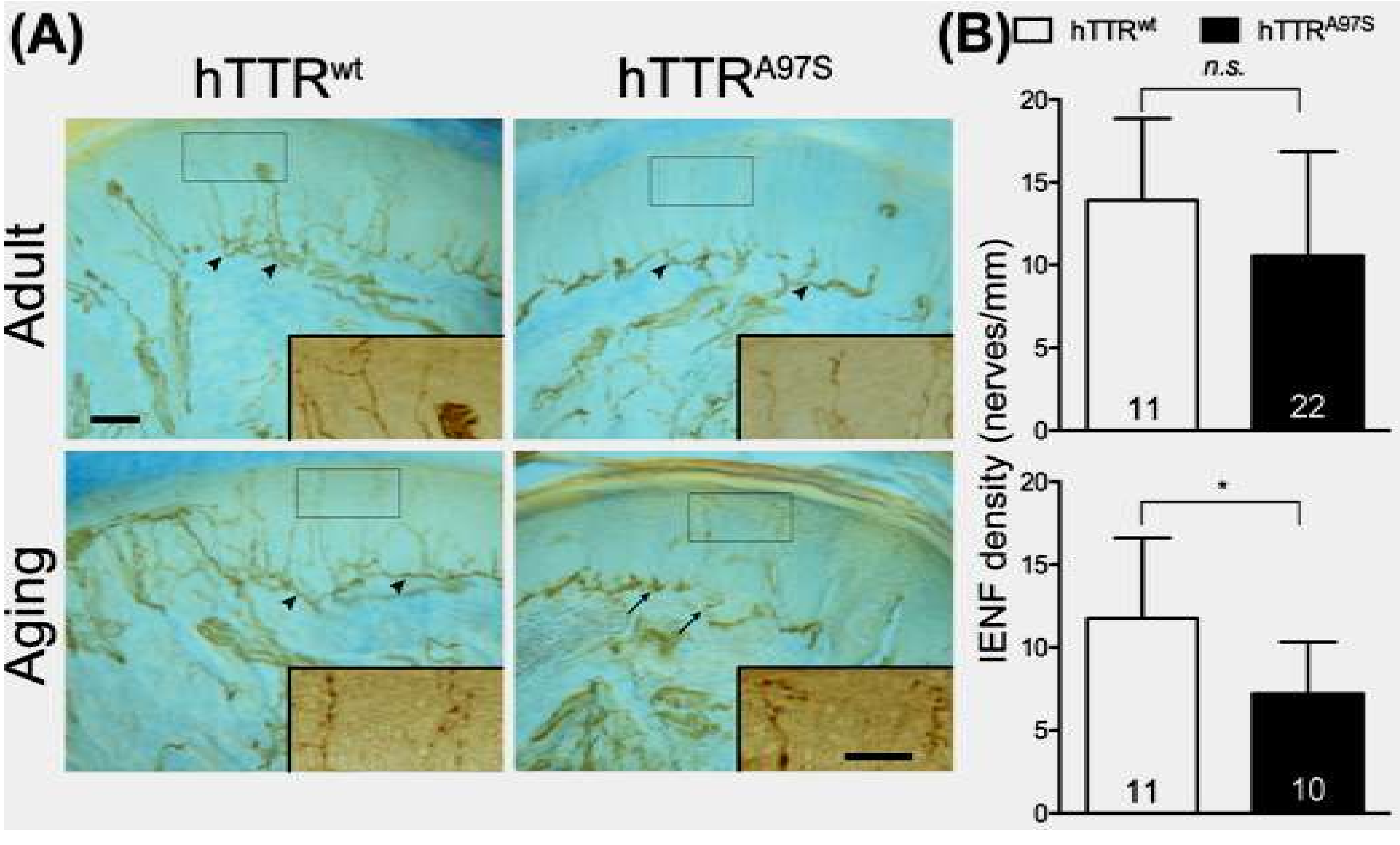
Amyloid deposites in knock-in mice

- survey: nerve, heart, GI, kidney, liver, skin
- amyloids in kidney, sural nerve, and skin: Congo red, polarized microscopy, and thioflavin T



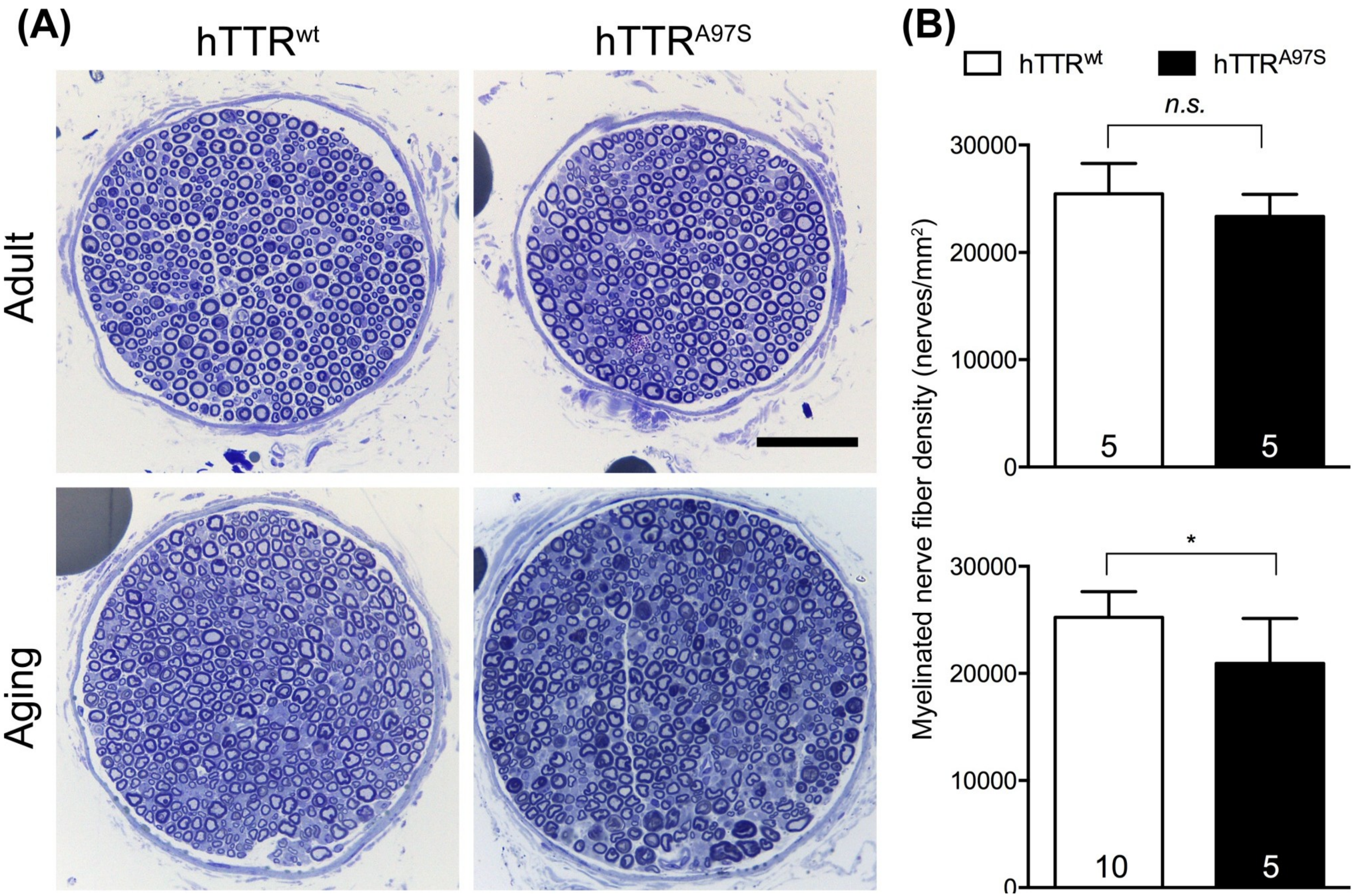
Sensory neuropathy (small fiber type): skin denervation in knock-in mice

- skin denervation, i.e. reduced intraepidermal nerve (IENF) density



Sensory neuropathy (large fiber type): Sural nerve pathology and morphometry

- reduced myelinated nerve fiber density in TTR-A97S mice

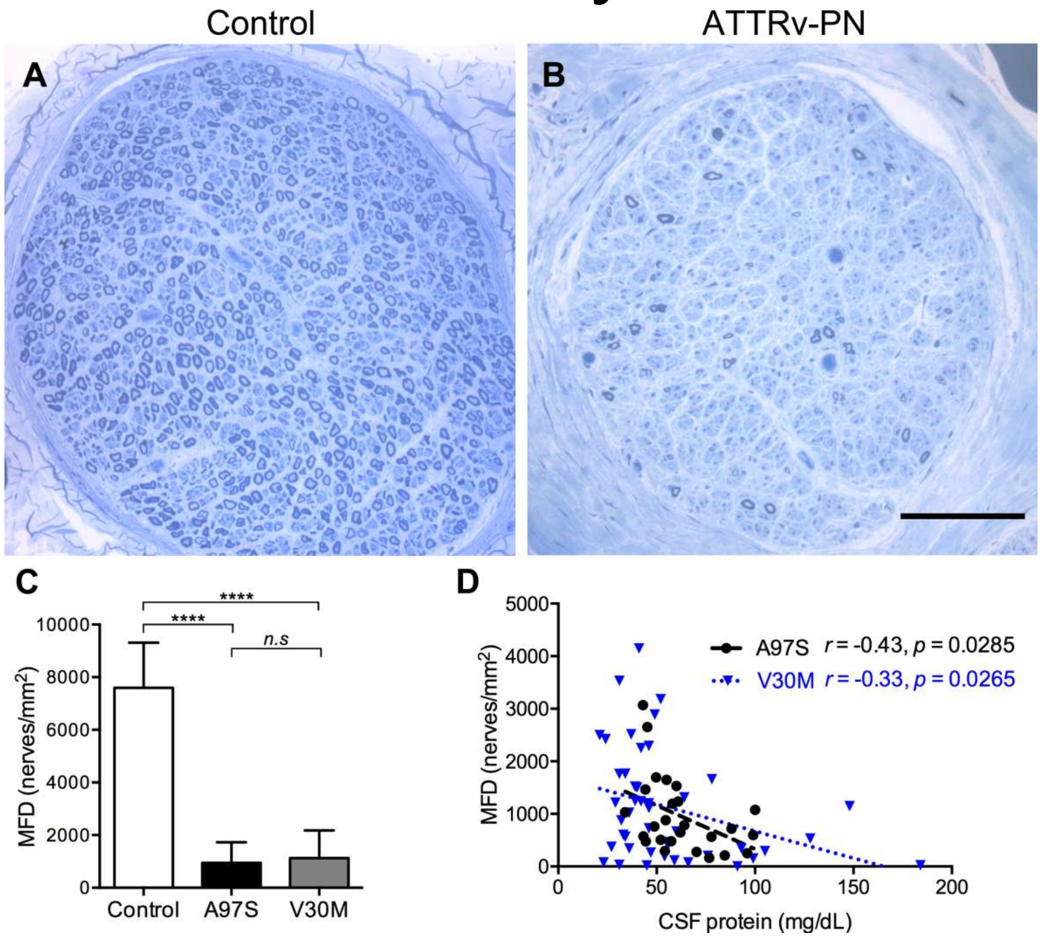


Mechanism of nerve degeneration in ATTRv

- Amyloid hypothesis: mainstream mechanism
  - Independent of amyloid deposition
- Aamyloid-independent mechanisms
  - Microangiopathy and thromboinflammation (Yeh, 2023)
  - Direct toxicity of TTR variant protein
  - ? Neuroinflammation (Chao, 2025)

CSF protein and nerve fiber density

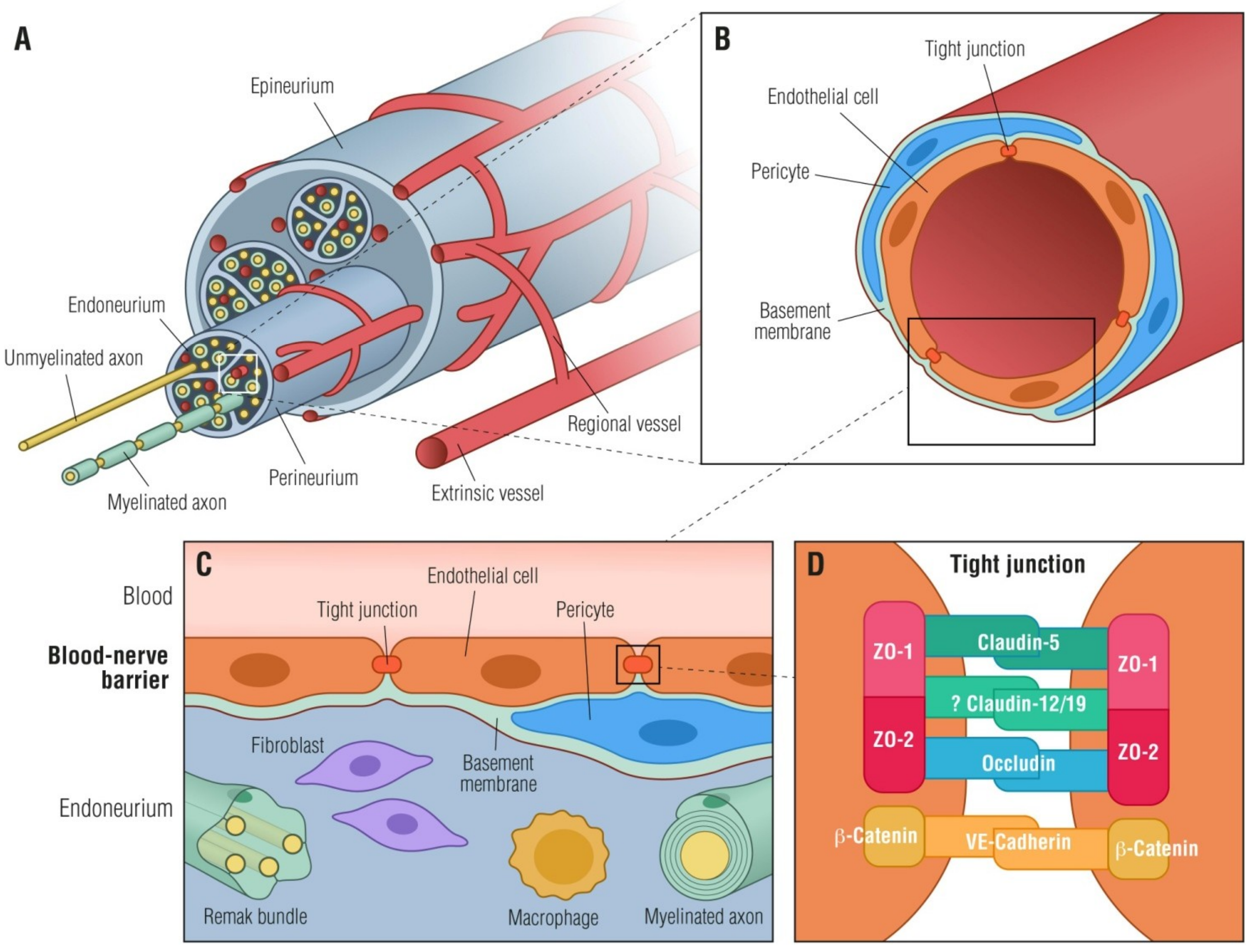
- Inverse relationship between CSF protein and nerve fiber density



(Chao et al, Brain, 2025)

blood-nerve barrier

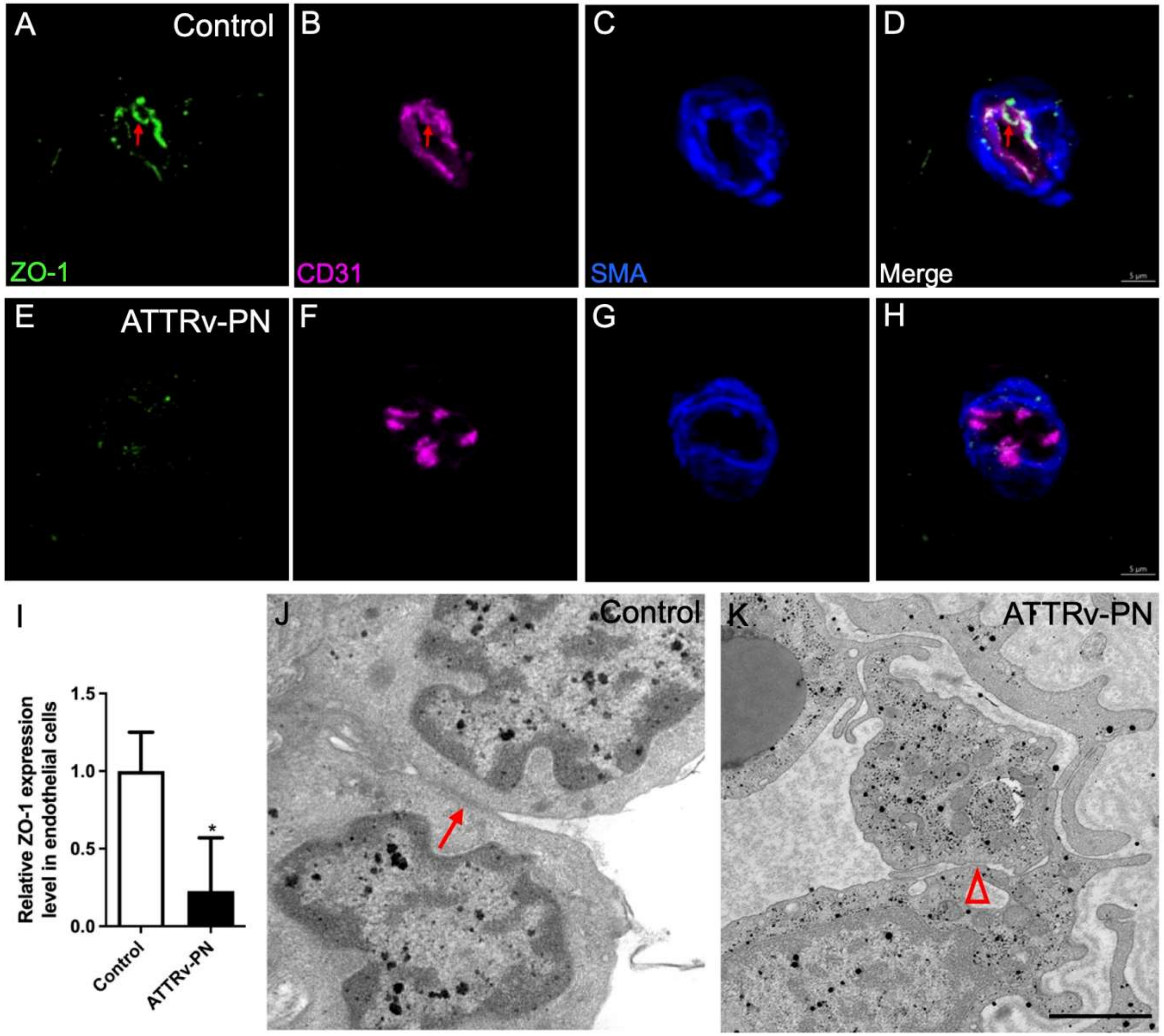
- tight junction molecules



(Richner et al. Front Neurosci 12:1038, 2019)

Impaired blood-brain nerve barrier in ATTRv

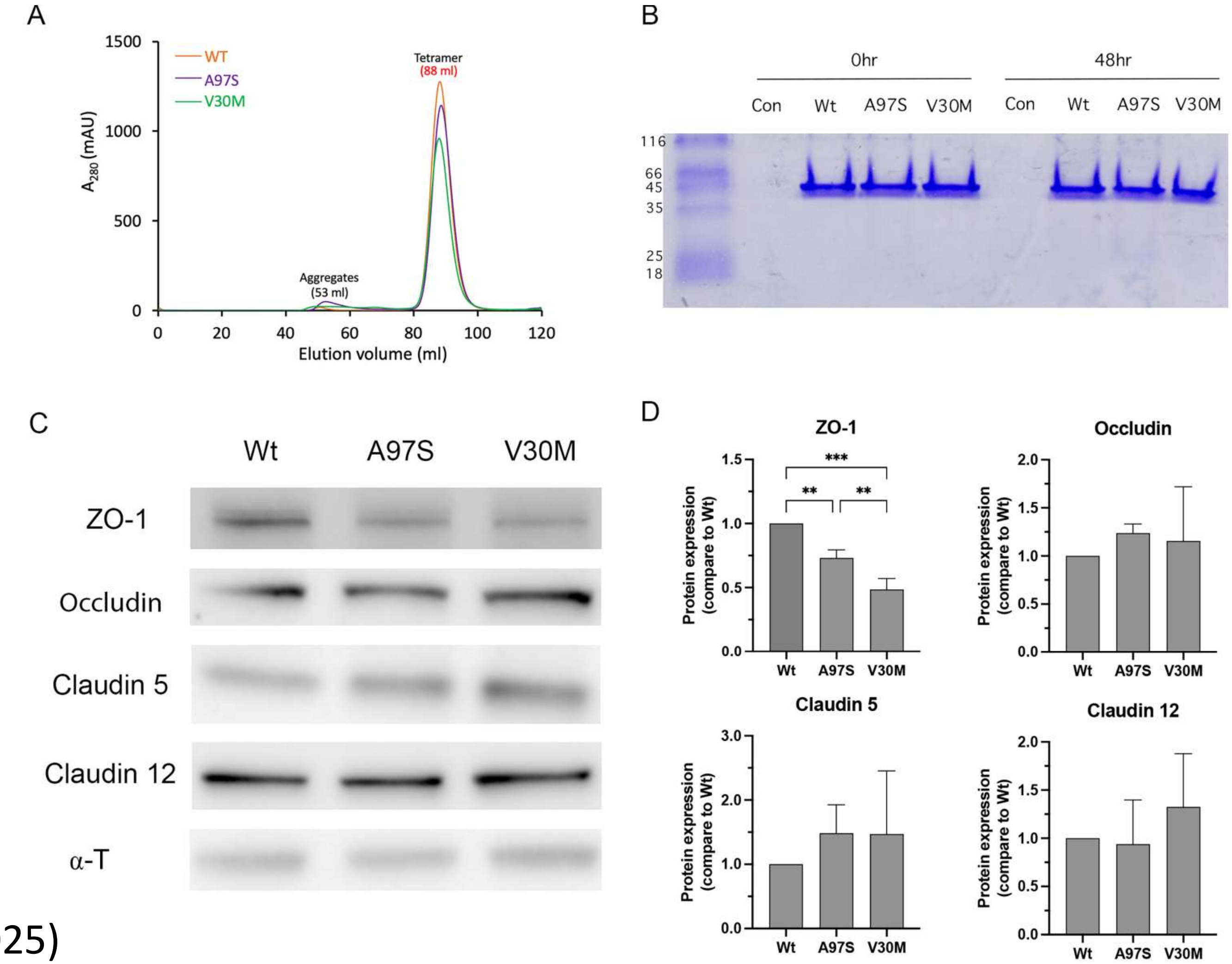
- Reduced expression of tight junction protein ZO-1 in ATTRv



(Chao et al, Brain, 2025)

Endothelial toxicity of TTR variant

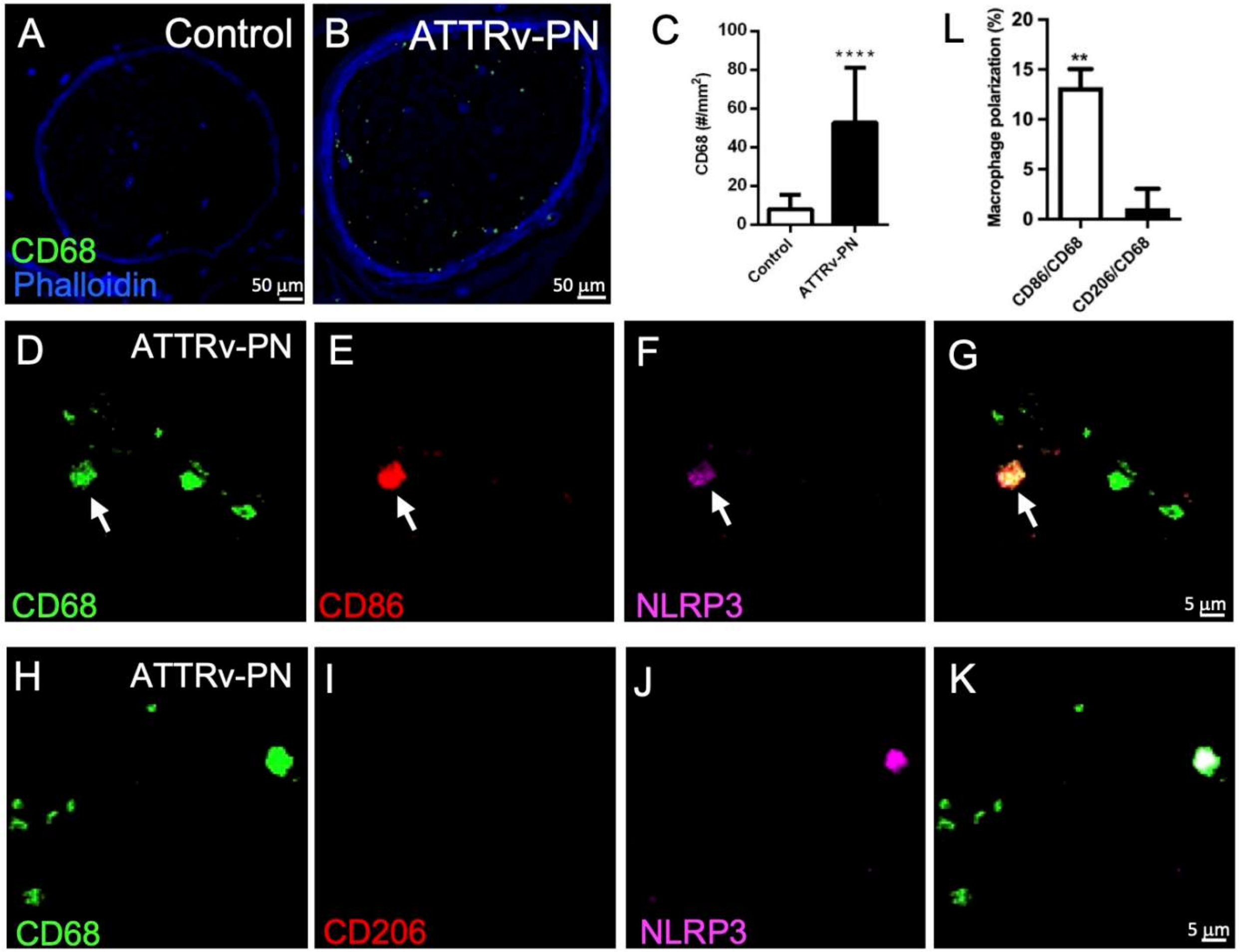
- Reduced ZO-1 expression when endothelium exposed to TTR-A97S and TTR-V30M



(Chao et al, Brain, 2025)

Neuroinflammation in ATTRv

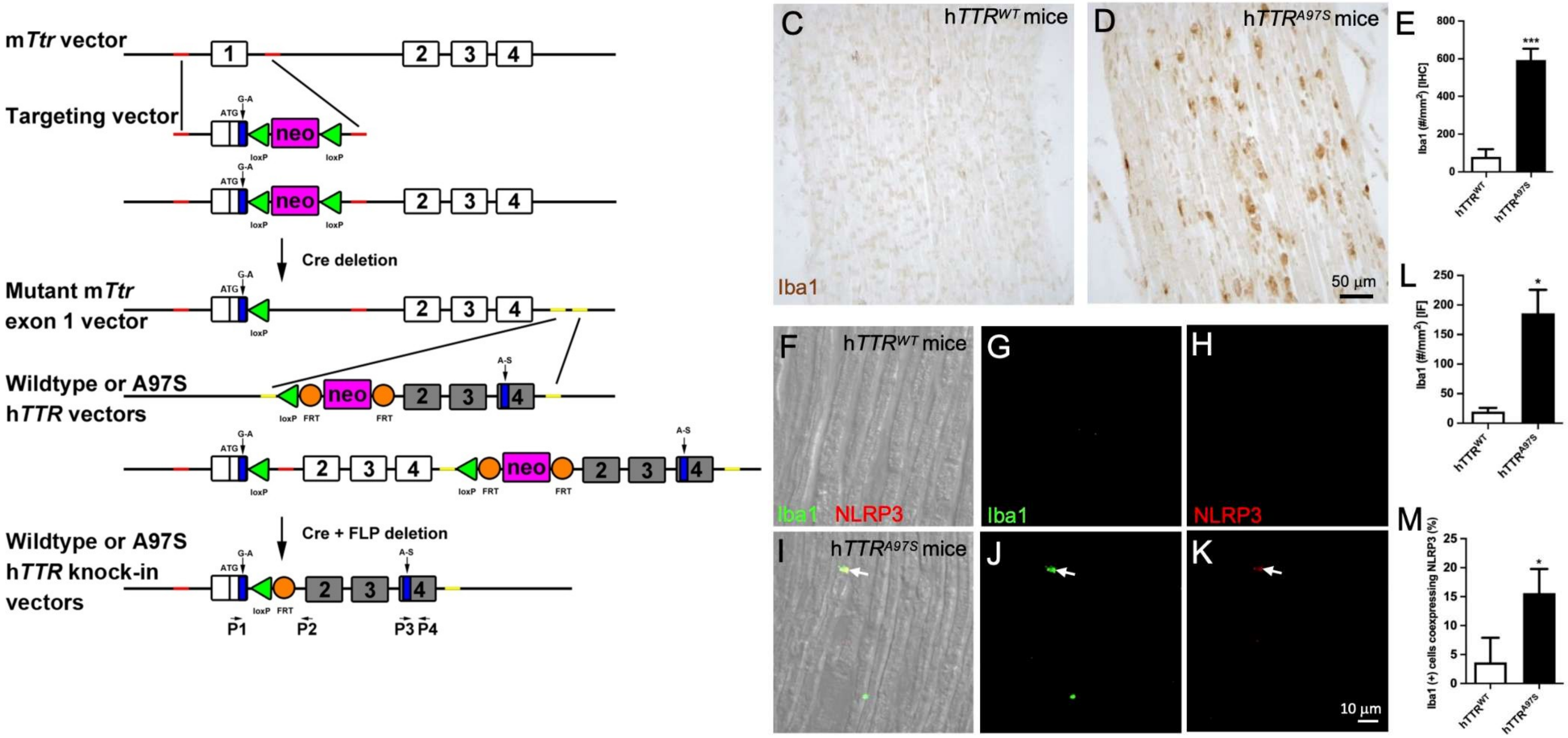
- Increased macrophage infiltration and inflammasome expression in ATTRv nerve



(Chao et al, Brain, 2025)

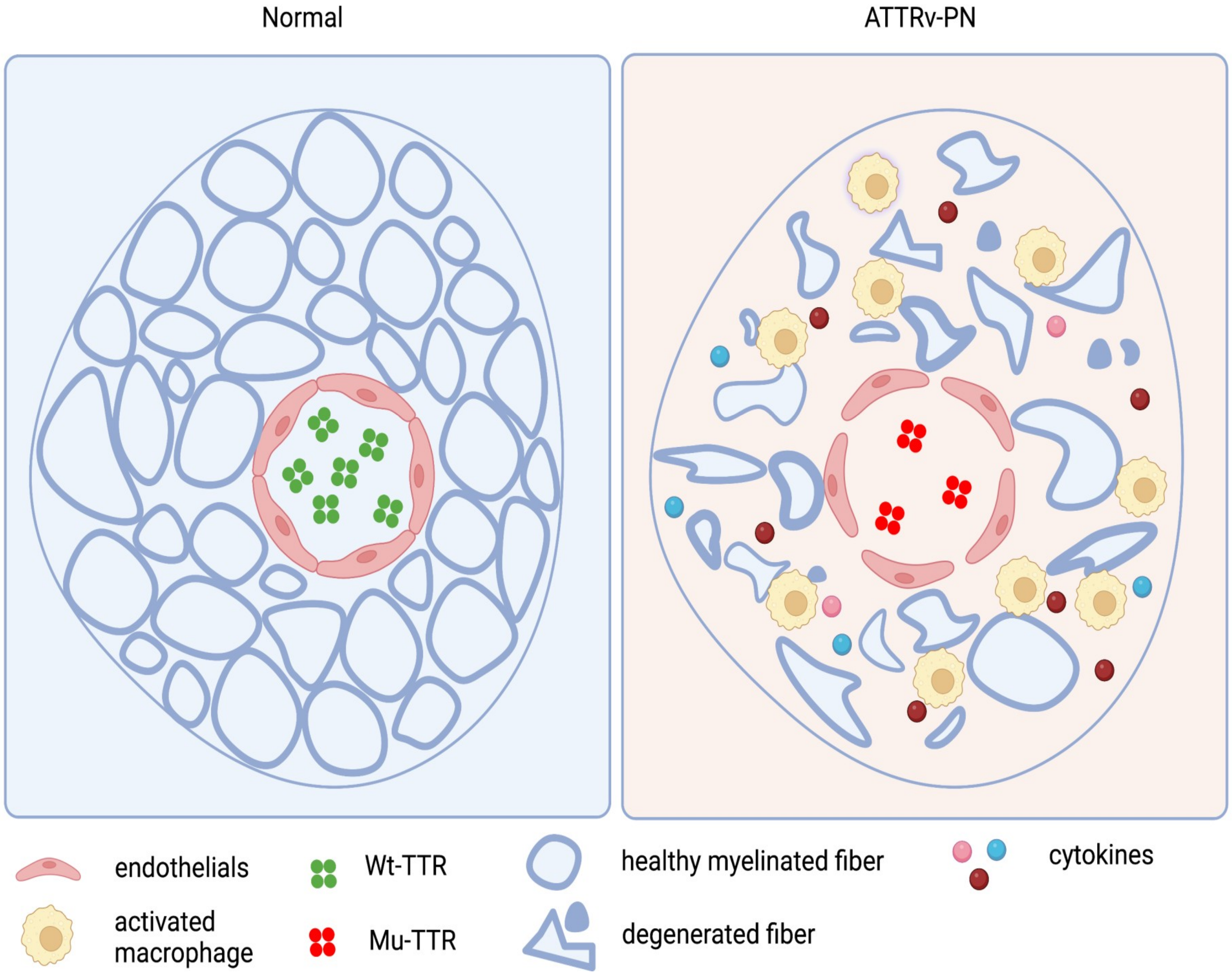
Neuroinflammation in A97S knock-in mice

- Macrophage infiltration and inflammasome expression in A97S knock-in mice



(Chao et al, Brain, 2025)

Impaired blood-nerve barrier as the initial event



Hereditary transthyretin amyloidosis (ATTRv) (p.Ala117Ser, A97S) in Taiwan

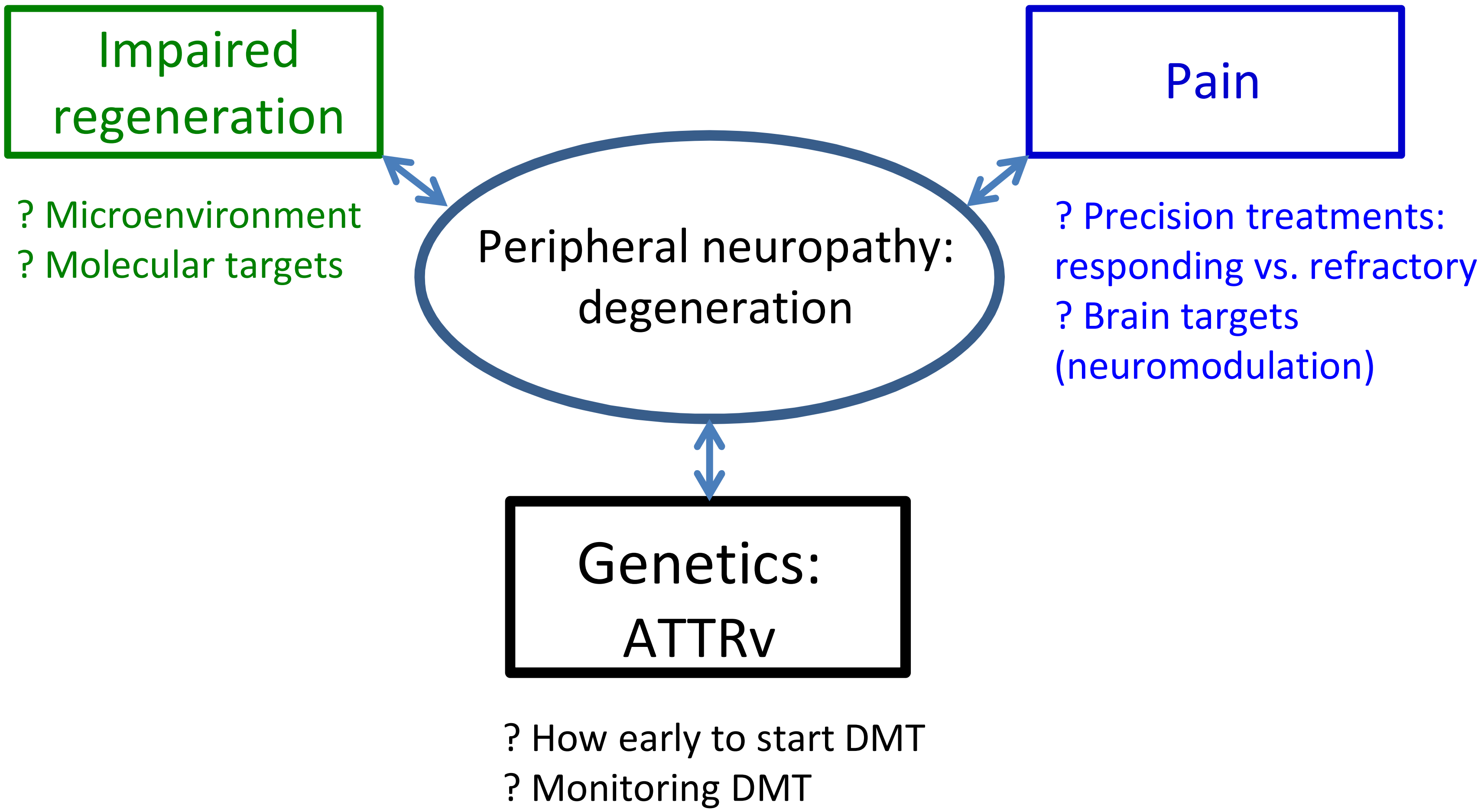
- The most common genetic neuropathy of late-onset with pan-modality involvement and axonal degeneration in Taiwan
- Some exhibited rapid decline on a background of slowly progressive course
  - Amyloid-independent mechanism: disruptionof blood-nerve barrier followed by neuroinflammation
- From an incurable disease to a treatable disorder: disease-modifying therapy (DMT)
  - Stabilizer: diflunisal, tafamidis, tolcapone
  - Silencing: RNAi (Patisiran), antisense oligonucleotide (ASO, inotersen)
- Emerging issues in the horizon of new ATTRv era
  - Continuous progression despite DMT
  - Early intervention

(Chao et al, Brain, 2025)

Emerging issues in ATTRv

- Biomarkers of disease and treatments
  - Onset: symptoms or abnormal examinations
  - Disease progression (Chao, 2019)
- Intervention
  - Choices of disease-modifying therapies (Chao, 2024)
    - Efficacy and Economy
  - Assessments of DMT
  - When to initiate therapy
    - Task force of EU neurological societies
- Ongoing degeneration
  - Clinical trial

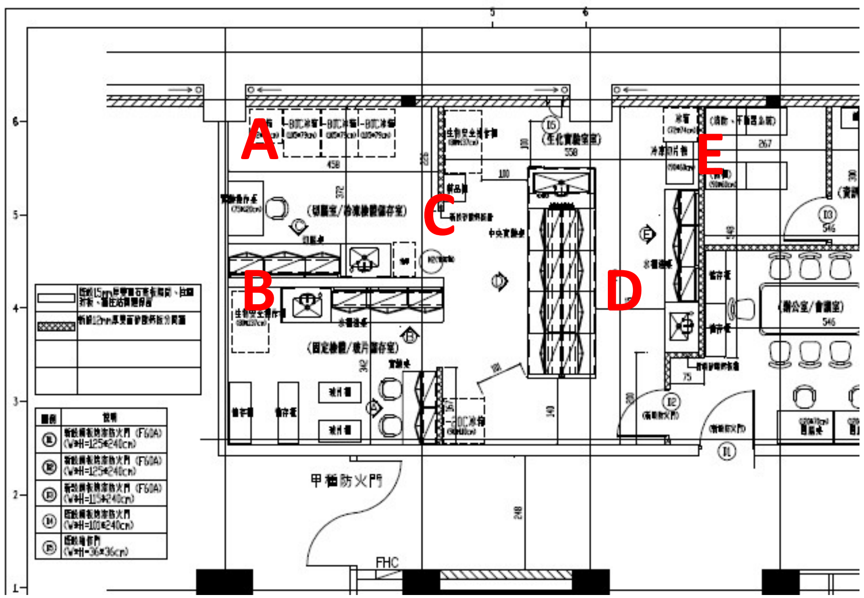
Small fiber neuropathy: degeneration and pain



「大愛的生物科學計畫」
(A project of Love and Biomedical Science)

台灣腦庫 (Taiwan Brain Bank)

- National Taiwan University College of Medicine B2
- budget: NTD $750 million
- capacity: 150 brains
  - Rare disease
  - Neurodegenerative disease
  - Control
- Freezer (A)、Pathology (B)、Lab (C)、Administration (D)、Information (E)



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社團法人 臺灣腦庫協會 Taiwan Brain Bank Association

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