# *Vascular Ehlers-Danlos syndrome: from men to mice*

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Centrum Medische Genetica

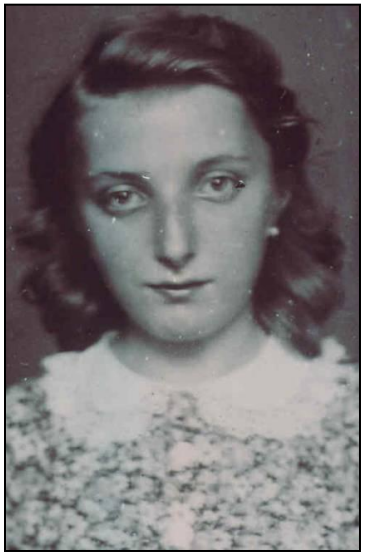
# EDS: Villefranche Classification 1997

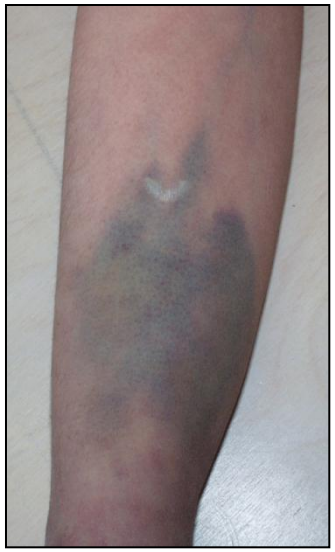
| EDS-Type | Inheritance pattern | Protein/Enzyme |
|---|---|---|
| Classic | AD | Procollagen type V |
| Hypermobility | AD | ? |
| Vascular | AD | Procollagen type III |
| Kyphoscoliotic | AR | Lysyl hydroxylase |
| Arthrochalasis | AD | Procollagen type I |
| Dermatosparaxis | AR | Procollagen I-N-proteinase |

*Beighton et al, AJMG, 1998*

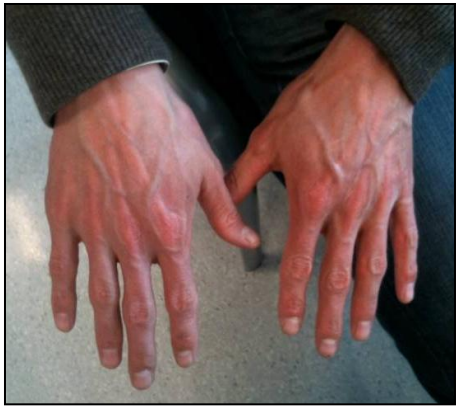
# EDS vascular type: clinical features

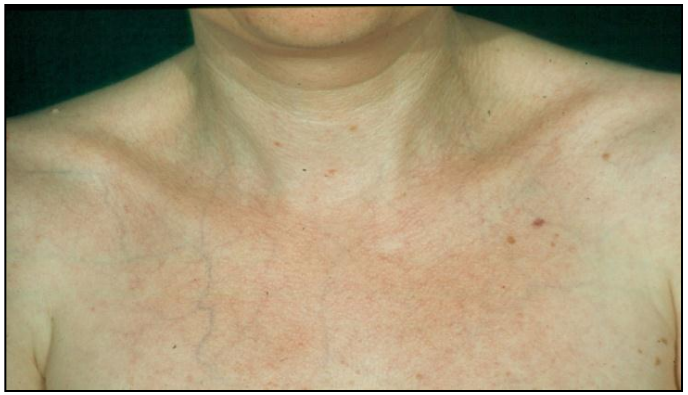
- Characteristic facial appearance
- Excessive bruising
- Thin, translucent and fragile skin
- Acrogeria
- Propensity to rupture of arteries and hollow organs at young age





- Caused by defects in type III collagen (*COL3A1*)





# EDS vascular type: Villefranche criteria for diagnosis

## Major diagnostic criteria:

- Thin translucent skin
- Arterial/ intestinal/ uterine fragility or rupture
- Extensive bruising
- Characteristic facial appearance

## Minor diagnostic criteria:

- Acrogeria
- Hypermobility of small joints
- Tendon and muscle rupture
- Talipes equinovarus
- Early-onset varicose veins
- Arteriovenous, carotid-cavernous fistel
- Pneumothorax/pneumohemothorax
- Gingival recession
- Positive family history, sudden death in a close relative

*Beighton et al AJMG 1998*

But the clinical presentation can be subtle!

## The Ghent experience

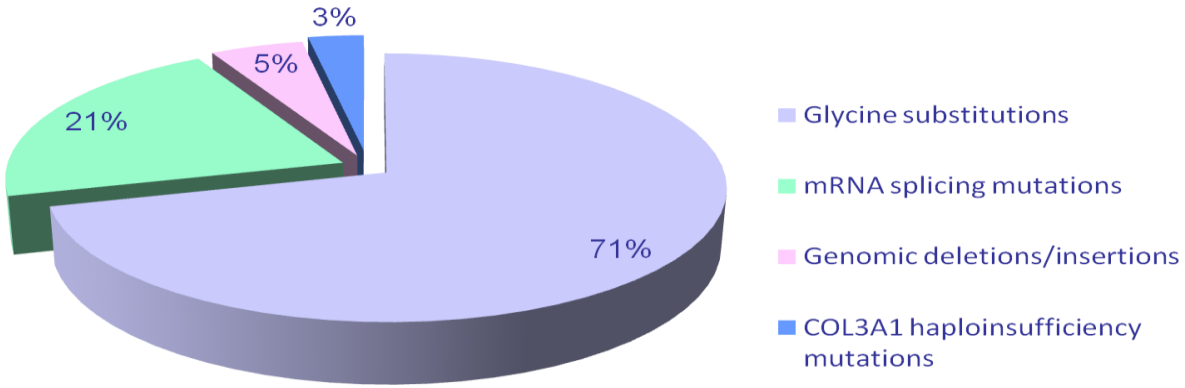
- Between 1985-2010: ~500 independent requests for type III collagen testing

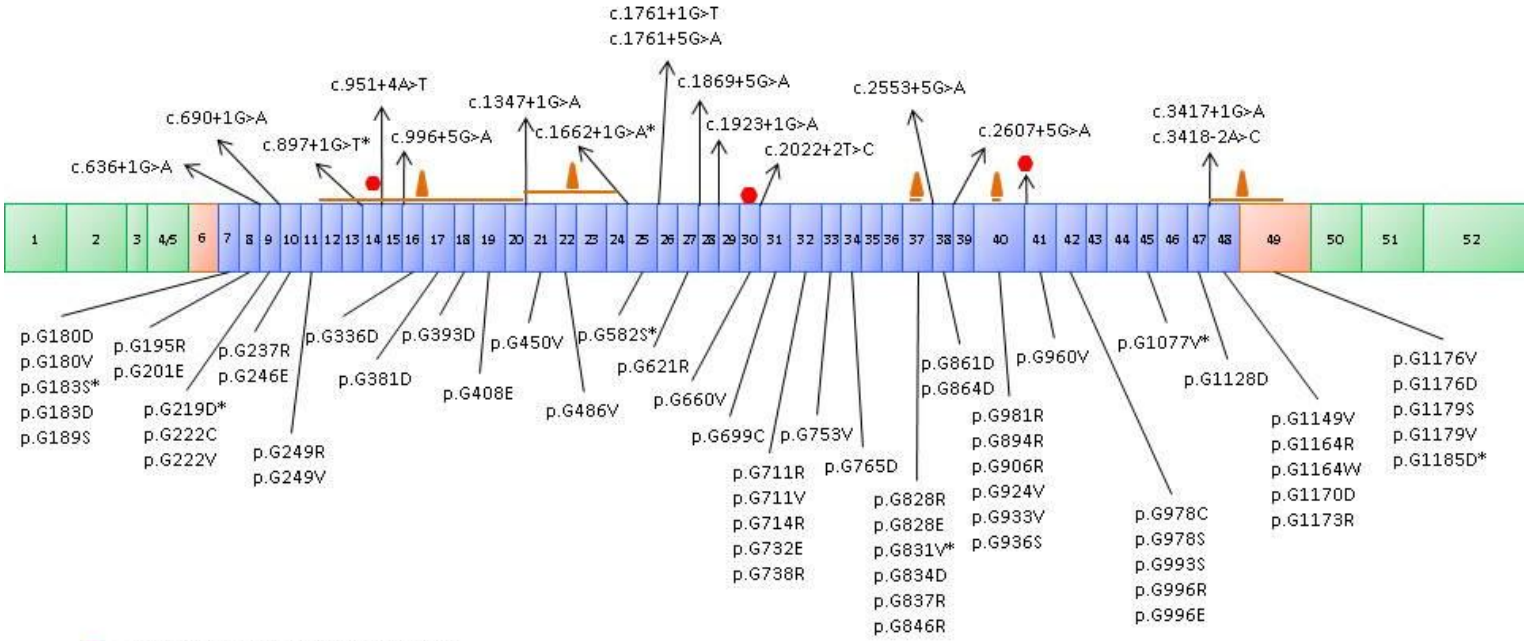
- Biochemical analysis of type III collagen performed in all patients

- Molecular analysis of *COL3A1* performed in 212 probands fulfilling Villefranche criteria either abnormal (99/212) or normal (112/212) biochemical result

# The Ghent experience: molecular results

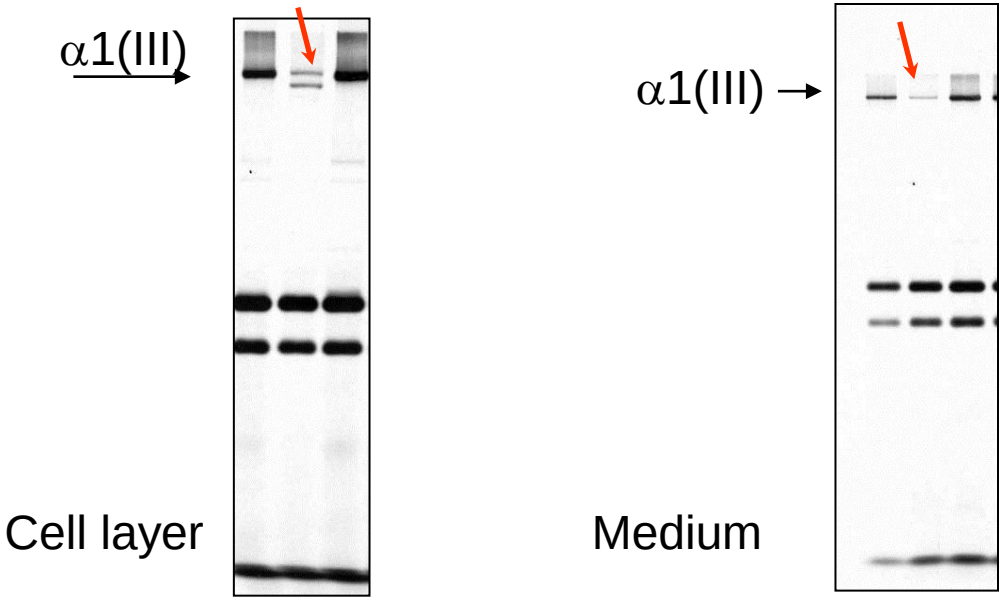
## *COL3A1* molecular analysis: 100/212 mutation in *COL3A1*



# The Ghent experience: Correlation of biochemical to molecular results

- 6/100 mutation-positive probands: normal biochemistry (sensitivity 94%)
- Normal biochemistry in all *COL3A1* haplo-insufficiency mutations
- 5/112 mutation-negative probands: abnormal biochemistry (specificity 95.5%)



# Clinical characteristics of the 100 *COL3A1* mutation-positive patients

- Reason for referral:
  - 60% referred after one (35%) or more (24%) major event(s)
  - 40% referred because of suspicious physical features (excessive bruising, translucent skin, acrogeria, facial appearance)

    → 16/40 family member(s) with Hx of major event or sudden death

- Age at time of ascertainment ranged between 4 and 74 yrs! (median 29 yrs)

- Survival
  - 22 patients deceased (median age: 33 yrs, range 15-56 yrs)
  - Major cause of death: arterial rupture (14/22), with or without pre-existing aneurysm
  - Bowel-rupture (n=1/22)
  - Post-surgical pulmonary embolism (n=1)
  - Cause of death undefined (n=6/22)

## Clinical characteristics of the 100 *COL3A1* mutation-positive patients

- Total number of major complications: n= 129 in 60 patients
  - 7% first major complication by age 20 yrs
  - 75% first for major complication by age 40 yrs
  - Majority (35/60) experienced more than 1 complication

- Arterial complications: 82 %
- Gastro-intestinal complications: 15%
- Pregnancy-related complications

  34 reported pregnancies in 21 women: major complications in 5/34 pregnancies
- Organ ruptures: 3% (spleen, liver)

# Clinical characteristics of the 100 *COL3A1* mutation-positive patients

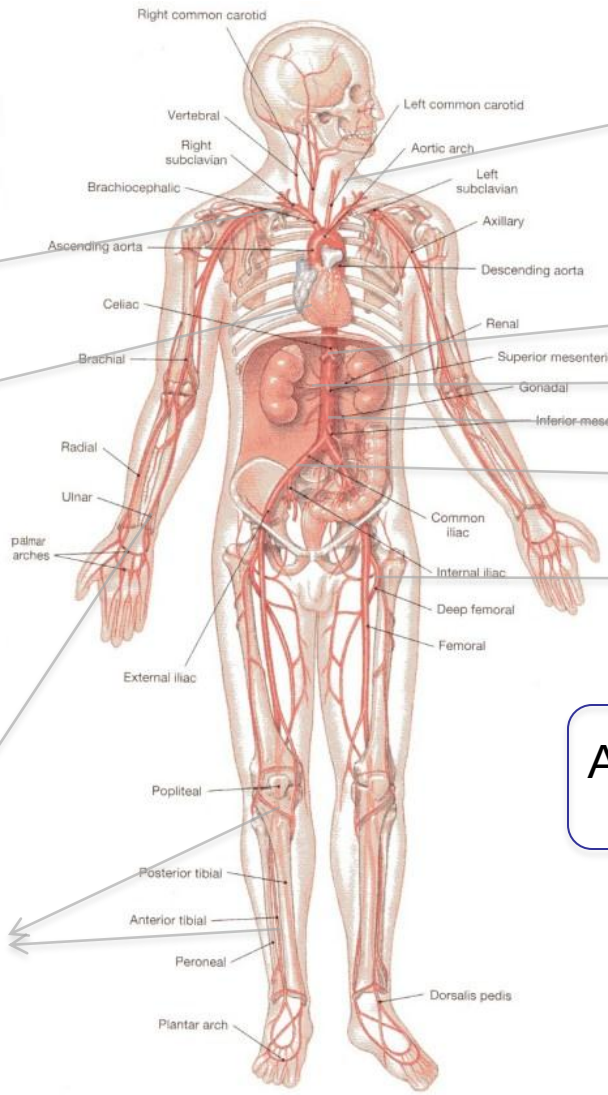
## Arterial complications: ~ 82% of all events

**Head and neck** 20%

- Carotis dissection/aneurysm
- Carotidocavernous fistula

**Thoracic vessels** 10%

- Subclavian
- Coronary




- Celiac
- Renal, Hepatic, Mesenteric
- Aorta
- Iliac
- Femoral

**Abdominal vessels/aorta** 55%

**Extremities** 15%

- Ulnar
- Popliteal/tibial

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# Clinical characteristics of the 112 *COL3A1* mutation-negative patients

- All presented combination of at least 2 of the major Villefranche criteria

- Reason for referral:
  - 45% one or more major event(s) (~85% vascular)
  - 15% arterial aneurysm, no dissection/rupture
  - 38% suspicious physical appearance
  - 2% suspicion battered child

- Survival:
  - only 1 patient deceased

- Total number of complications: n=81 in 65 patients
- 16/65 patients experienced more than 1 major event

# Clinical characteristics of the 112 *COL3A1* mutation-negative patients

## Arterial complications: ~ 92% of all events

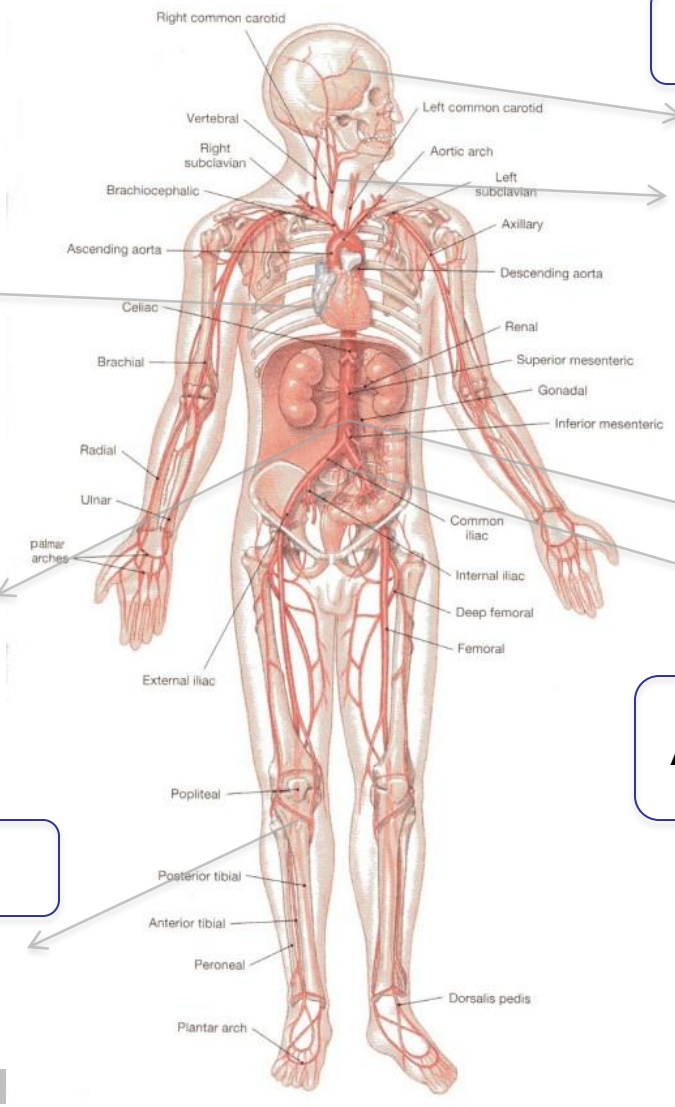
**Head and neck** 28%

Intracranial aneurysm/dissection

Carotis/vertebalis dissection/aneurysm

**Thoracic vessels** 6,5%

Coronary




**Aorta** 25,5%

Renal, Hepatic, Mesenteric

Iliac

**Abdominal vessels** 21%

**Extremities** 4%

Popliteal

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## Clinical characteristics of the 112 *COL3A1* mutation-negative patients

- Other molecular defects found in 7/112 patients
  - *TGFBR2*: n=1
  - *COL1A1/COL1A2:* n =4
  - *COL5A1*: n=1
  - *ACTA2*: n=1

- In 8 patients*: COL3A1* variant in coding region leading to substitution of a non-glycine residue
  - p.Arg271Gln, p.Glu370Lys, p.Pro602Thr, p.Ala679Thr, p.His1269His, p.Lys1313Arg

- No *COL3A1* mutation identified in ~60 patients with isolated dissection of carotid or vertebral artery

## Vascular EDS: a therapeutic challenge

- High risk for dramatic complications with reduced life expectancy
- Complications are unpredictable and sudden, no monitoring possible
- No causal therapy

- Therapeutic options
  - Surveillance
  - Avoidance of risk
  - Surgical treatment of complications

  - Beneficial effect of celiprolol in the prevention of arterial complications (Ong et al, Lancet, 2010). Start at which age?

# Preclinical investigation of vascular EDS been hampered by the lack of a suitable animal model

- Targeted ablation of *col3a1* gene (*Liu et al, PNAS, 1997*):
  - *col3a1*-/- mice:
    - 90-95% mortality (mostly within 48 hours of life)
    - 5-10% survive until adulthood, but die prematurely due to rupture of major blood vessels
  - *col3a1*+/- (*Cooper et al, Vet.Path, 2010*):
    - phenotypically normal, normal life span
    - subclinical phenotype of vascular fragility (reduced collagen content in abdominal aorta, diminished wall strength of aorta), age-dependent in expression

- Spontaneously generated mouse line (*Smith et al, Cardiovasc. Res., 2011*)
  - Spontaneous 185 deletion, including promoter region and exons 1-39 of *col3a1* (+/*col3a1*$^{\Delta}$)
    - Sudden, unexpected death from rupture of thoracic aorta
    - Median age 6 weeks
    - Incomplete penetrance
    - Sex ratio M:F, 2:1
    - Not associated with elevated blood pressure or aneurysm formation

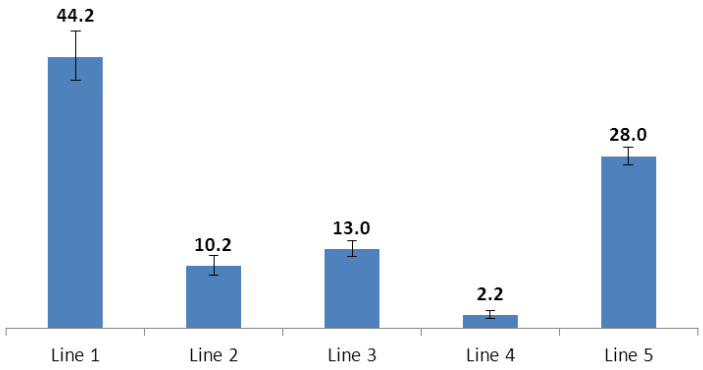
Limitation: haplo-insufficient mouse model, whereas most mutations in human vEDS have a dominant negative effect

# Generation of a vEDS mouse model using a transgenic approach

- Engineered BAC containing the full *col3a1* gene with a p.Gly183Ser mutation, and its own promoter, 5’ and 3’ UTR and regulatory regions, fluorescent reporter gene sequence, kanamycine/neomycine cassette between two loxP sites

- Injected into C57BL/6 fertilized eggs and placed into pseudopregnant mice – random intergration of one or more copies into the genome

- Transgene copy number determined by AS-PCR

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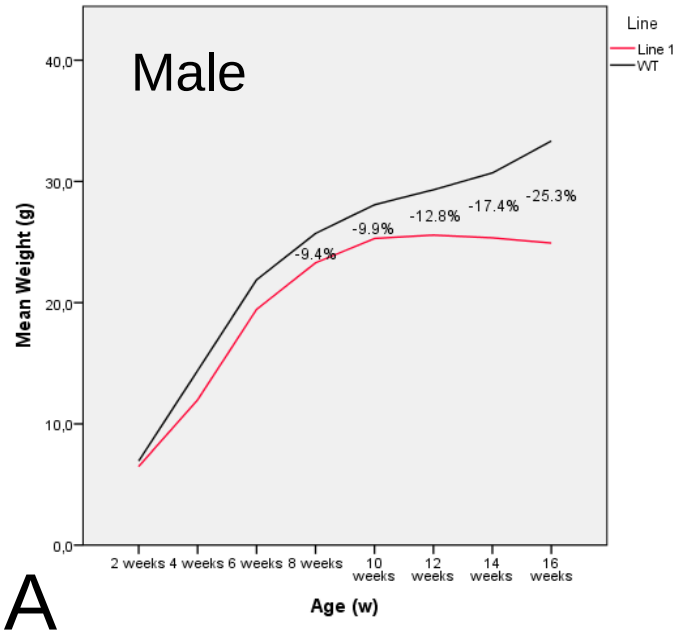
- Gross phenotype:

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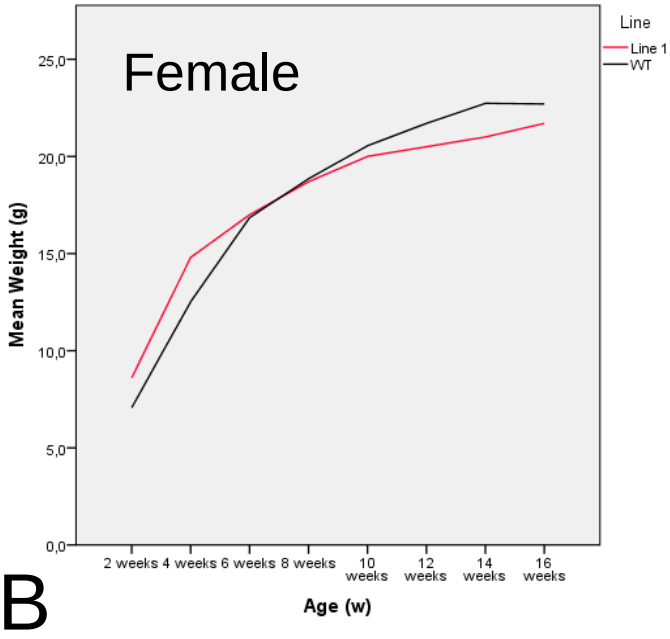


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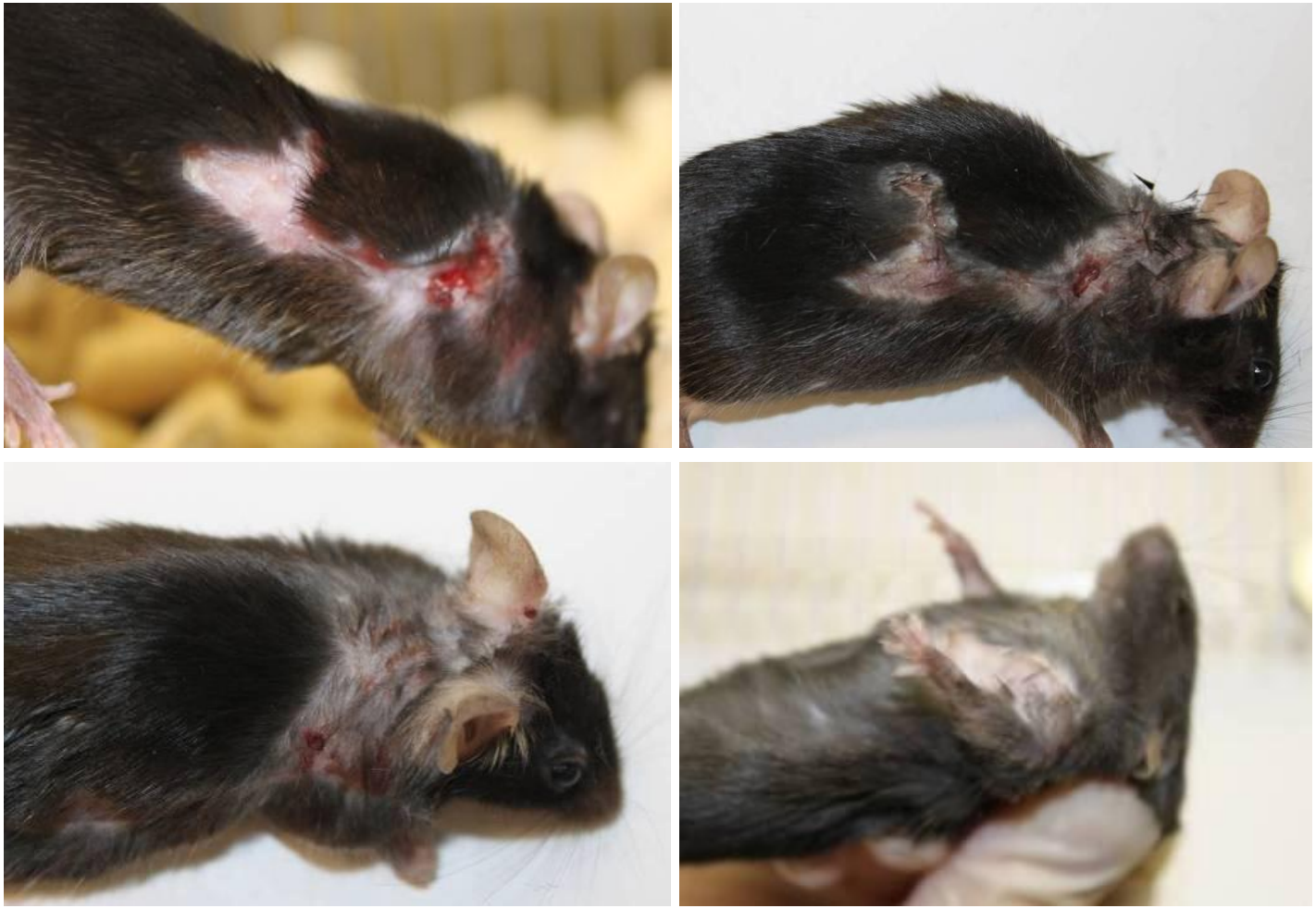
Adult male mice in *Col3a1* transgenic mouse line 1 are significantly smaller than strain-, age-, and sex-matched WT C57BL/6 controls

# Transgenic mouse model



- Gross phenotype:
  - Thin and fragile skin is noted during dissection of the euthanized mice (line 1)
  - Preliminary vascular corrosion casting experiments on euthanized *Col3a1* transgenic mice (line 1) were complicated by rupture of thoracic arterial vessels, suggesting an increased fragility of the vascular system
  - No detectable differences in the heart and large arteries between *Col3a1* transgenic mice and WT mice by echocardiography.

## Future studies

- Microscopy of skin and vascular walls (collagen fiber patterns, elastic fiber fragmentation
- Biomechanical testing of skin, colon and vascular wall
- Study of the vasculature
  - 3D reconstruction of vascular structures
  - Micro CT
- Immunohistochemistry and qPCR to evaluate TGFbeta and adrenoreceptor downstream targets
- testing of therapeutic agents

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