Vascular Ehlers-Danlos syndrome: from men to mice

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<thead>
<tr>
<th>EDS-Type</th>
<th>Inheritance pattern</th>
<th>Protein/Enzyme</th>
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<tr>
<td>Classic</td>
<td>AD</td>
<td>Procollagen type V</td>
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<tr>
<td>Hypermobility</td>
<td>AD</td>
<td>?</td>
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<tr>
<td>Vascular</td>
<td>AD</td>
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<tr>
<td>Kyphoscoliotic</td>
<td>AR</td>
<td>Lysyl hydroxylase</td>
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<td>Arthrochalasis</td>
<td>AD</td>
<td>Procollagen type I</td>
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<tr>
<td>Dermatosparaxis</td>
<td>AR</td>
<td>Procollagen I-N-proteinase</td>
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*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)
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

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 \textit{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

**Thoracic vessels** 10%
- Subclavian
- Coronary

**Head and neck** 20%
- Carotis dissection/aneurysm
- Carotidocavernous fistula

**Extremities** 15%
- Ulnar
- Popliteal/tibial

**Abdominal vessels/aorta** 55%
- Celiac
- Renal, Hepatic, Mesenteric
- Aorta
- Iliac
- Femoral
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 \textit{COL3A1} mutation-negative patients

Arterial complications: \(\sim 92\%\) of all events

- **Thoracic vessels** 6.5%
  - Coronary

- **Aorta** 25.5%

- **Extremities** 4%
  - Popliteal

- **Head and neck** 28%
  - Intracranial aneurysm/dissection
  - Carotis/vertebral dissection/aneurysm

- **Abdominal vessels** 21%
  - Renal, Hepatic, Mesenteric
  - Iliac
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

- 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 has 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
Mouse models

- **Spontaneously generated mouse line** (*Smith et al, Cardiovasc. Res., 2011*)
  - Spontaneous 185 deletion, including promoter region and exons 1-39 of *col3a1* (+/col3a1Δ)
    - 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 integration of one or more copies into the genome

- Transgene copy number determined by AS-PCR
Transgenic mouse model

- Gross phenotype:

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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