Genetic
Girls, families....

1. The aim of this study was:
   - identification of gene(s) responsible of idiopathic scoliosis,
   - identification of mutations,
   - study of correlation between genotype and phenotype

2. Analysis of the genetic aspects in families with idiopathic scoliosis

Nancy Miller, M.D.,
John Hopkins Hospital, (Baltimore, Maryland, USA)

Genetic

Methods. Model-independent linkage analysis of qualitative and quantitative traits related to scoliosis was used to screen genotyping data from 391 markers in 202 families (1198 individuals).

Subsets of families with probands having a curve $\geq 30^\circ$ were dichotomized based on the most likely mode of inheritance for each family (autosomal dominant or X-linked dominant). Fine mapping was performed to show linkage to candidate regions on chromosome 19.

Results. When the threshold of disease was set at a curvature of $\geq 30^\circ$, qualitative linkage analysis revealed significant results at 2 successive markers on chromosome 19.

Conclusion. The data confirm a previously reported genetic locus on chromosome 19 as potentially significant in the etiology of idiopathic scoliosis.

Genetic

Dr Carol Wise, Texas Scottish Rite Hospital for Children, Dallas, Etas-Unis

• How to locate and identify the genes which are in relation with the discovery of an idiopathic scoliosis?

Linkage between scoliosis and chromosome 4q, 6p, distal 10q, and 18q within one large extended family

Dermatoglyphics were compared in 114 female control individuals and 164 female patients with AIS (minimum Cobb angle = 10°).

Significant asymmetry suggesting both:

- Developmental instability
- Directional asymmetry

Growing corticospinal and somatosensory axons cross the midline in the medulla to reach their targets and thus form the basis of contralateral motor control and sensory input.

The motor and sensory projections appeared uncrossed in patients affected with HGPPS, we identified mutations in the ROBO 3 gene, which shares homology with roundabout genes important in axon guidance in developing Drosophila, Zebrafish, and mouse.

Genetic

Salt Lake City

145 AIS  →  100 from families known to have members affected with AIS

45  →  14 not found in the genealogic database

31  →  27 connected to at least one of the scoliotic family

127 / 131

Thillard MJ. Déformations de la colonne vertébrale consécutives à l’epiphysectomie chez le poussin. Extrait des comptes rendus de l’association des anatomistes 1959; 751-8

Dubousset J, Queneau P, Thillard MJ. Experimental Scoliosis Induced by pineal and diencephalic lesions in young chickens; its relation with clinical findings. Orthop Trans 1983; 7:7.
Melatonin

Professor Masafumi Machida
Nihon University School of Medicine
Tokyo, Japon
Study the role of melatonin in the origin and progression of the scoliosis


Melatonin

The prime function of the pineal gland in chicken is the production of melatonin.

The effect of pinealectomy in causing scoliosis in chickens has therefore been attributed to melatonin deficiency.


Machida M, Miyashita Y, Murai I and al. Role of serotonin for scoliotic deformity in pinealectomized chicken.
Melatonin

Thillard MJ. Déformations de la colonne vertébrale consécutives à l’epiphysectomie chez le poussin. Extrait des comptes rendus de l’association des anatomistes 1959; 751-8

Coillard C, Badeaux J, Rivard C. Vertebral deformation and scoliosis. Proceedings of the Québec Scoliosis Society, 1994; 24


## Melatonin

<table>
<thead>
<tr>
<th>Author</th>
<th>Year of publication</th>
<th>%Scoliosis produced</th>
<th>Number of Scoliosis/Number of chickens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thillard</td>
<td>1959</td>
<td>66%</td>
<td>33/50</td>
</tr>
<tr>
<td>Mashida</td>
<td>1993</td>
<td>100%</td>
<td>30/30</td>
</tr>
<tr>
<td>Coillard</td>
<td>1994</td>
<td>80%</td>
<td>20/25</td>
</tr>
<tr>
<td>Kanemura</td>
<td>1997</td>
<td>68%</td>
<td>17/25</td>
</tr>
<tr>
<td>Wang</td>
<td>1997</td>
<td>60%</td>
<td>18/30</td>
</tr>
<tr>
<td>Wang</td>
<td>1998</td>
<td>52%</td>
<td>17/33</td>
</tr>
<tr>
<td>O’Kelly</td>
<td>1999</td>
<td>48%</td>
<td>10/21</td>
</tr>
<tr>
<td>Bagnall</td>
<td>2001</td>
<td>55%</td>
<td>54/98</td>
</tr>
</tbody>
</table>
Melatonin

109 Chickens

Group 1 (9) : Control group. No surgery.

Group 2 (20) : Only the skull + 3 sinuses cut

Group 3 (20) : Sham procedure, touched but not removed

Group 4 (25) : Just cut the pineal stalk

Group 5 (35) : Complete pinealectomy

Melatonin

Beuerlein M, Wilson J, Moreau M and al. The critical stage of pinealectomy surgery after which scoliosis is produced in young chickens.


Group 4 (25) : Just cut the pineal stalk 68%

Group 5 (35) : Complete pinealectomy 60%

Same amount of decrease in serum melatonin level

% SCOLIOSIS
35 Chickens → Control group with no surgery 10

25 had a pinealectomy

13 (52%) had a scoliosis

All scoliosis were thoraco-lumbar with lordosis, rotation and wedging

The thoracic curve above has the appearance of a compensatory curve

Severity of pelvic wing deformity correlated to the Cobb angle

Mashida showed that *intra peritoneal injection of melatonin prevents the development of scoliosis in pinealectomized chickens* that otherwise would have developed scoliosis.

After pinealectomy Mashida autografted the removed pineal gland into the thoracic musculature of the chickens.

<table>
<thead>
<tr>
<th></th>
<th>%Scoliosis produced</th>
<th>Nber of Scoliosis/Nber of chickens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinealectomy</td>
<td>100%</td>
<td>30/30</td>
</tr>
<tr>
<td>Pinealectomy + autografting</td>
<td>10%</td>
<td>3/30</td>
</tr>
<tr>
<td>Surgical Procedure</td>
<td>Number of chickens</td>
<td>Killed at 1 week</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>--------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Pinealectomy</td>
<td>43</td>
<td>8</td>
</tr>
<tr>
<td>Cut stalk</td>
<td>43</td>
<td>5</td>
</tr>
<tr>
<td>Pinealectomy + transplantation</td>
<td>35</td>
<td>10</td>
</tr>
<tr>
<td>Control</td>
<td>25</td>
<td>11</td>
</tr>
</tbody>
</table>

*Bagnall K, Beuerlein M, Johnson P and al. Pineal transplantation after pinealectomy in young chickens has no effect on the development of scoliosis. Spine 2001; 26: 1022 - 27*
# Melatonin

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Number of chickens</th>
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<td>25</td>
<td>11</td>
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Average peak melatonin level: Significantly

Bagnall 2001
# Melatonin

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<tr>
<td>Control</td>
<td>25</td>
<td>11</td>
</tr>
</tbody>
</table>

Average peak melatonin level did not increase.

[Bagnall 2001]
Melatonin

In mammals transplantation to the anterior chamber of the eye is the only effective means to reestablish pineal function.

Similar results might be expected in avian species.

Most scoliosis develops in pinealectomized chickens within the first 2 weeks after surgery.

Mashida could not produce the same result with rats and concluded that the bipodal condition may play an important role in the development of scoliosis.

Pinealectomy in rats maintained in a bipodal condition produces a scoliosis.
Melatonin

Pinealectomy in

4 sham operation → No scoliosis

10 quadrupedal rats → No scoliosis

20 bipedal rats → 100% scoliosis

10 bipedal + melatonin pellets 100 mg per 90 days release

10% scoliosis

Melatonin


Melatonin

Docteurs Alain Moreau and Hubert Labelle

Hôpital Sainte Justine Montréal, Québec, Canada

Analysis of molecular expression and neurological systematisation of melatonin
Melatonin

The idea was that a defect of melatonin signaling activity could generate effects similar to a melatonine deficiency.

Cellular model = Osteoblastes  
Bone is a tissue responding to melatonin  
Persistent osteopenia often reported in AIS  

AIS = 41, Non Idiopathic Scoliosis = 15, Control Group = 2

Osteoblasts from patients with AIS showed hardly any capability to inhibit forskolin-stimulated adenyl cyclase activity in response to melatonin, clearly indicating an impairment of melatonin signaling in AIS.

Melatonin signaling is clearly impaired in ostéoblasts of all patients with adolescent idiopathic scoliosis tested.

Classification of patients with adolescent idiopathic scoliosis in three groups based on functional in vitro assays suggests the presence of distinct mutations.

Comparison between AIS and congenital scoliosis groups showed no significant variation in those assays, suggesting a possible link between AIS and some type of congenital scoliosis.

Purkiss reported a high rate of AIS (17.3%) in families affected by congenital scoliosis.


Clin Orthop 2002; 27 - 31
Genetic and melatonin

Linkage between scoliosis and chromosome 4q, 6p, distal 10q, and 18q within one large extended family


In mammals two subtypes of melatonin receptors have been isolated: melatonin 1A and 1B

The gene for melatonin 1A is located on chromosome 4q

Genetic and melatonin

113 AIS and 64 non-affected patients

No evidence of linkage to chromosome 4q and no mutation in the coding region of the gene for human melatonin receptor

Disco-ligamentous concept

Jeremy Fairbanks, M.D. and Jill Urban, M.D. Oxford, UK

- Role of the « soft tissue »: fibroblasts and elastic fibers abnormalities?

Disco-ligamentous concept

Control discs : 2 patients (1 tumor, 1 trauma)
Scoliotic discs : 6 patients (3 AIS, 3 neuromuscular scoliosis)

Abundant and organized network of elastic fibers in control group

Sparse and disrupted network in Scoliotic group

Could be involved in the progression of the spinal deformity

15 IVD in AIS were obtained intraoperatively
17 IVD from normal spines obtained post-mortem

<table>
<thead>
<tr>
<th></th>
<th>Anulus</th>
<th>Nucleus</th>
<th>End plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total collagen content</td>
<td>S \</td>
<td></td>
<td>S \</td>
</tr>
<tr>
<td>Glycosaminoglycan</td>
<td></td>
<td>S \</td>
<td>S \</td>
</tr>
<tr>
<td>Total protein content</td>
<td></td>
<td></td>
<td>S \</td>
</tr>
<tr>
<td>Water content</td>
<td>S \</td>
<td></td>
<td>S \</td>
</tr>
</tbody>
</table>


15 IVD in AIS were obtained intraoperatively
17 IVD from normal spines obtained post-mortem

No differences Concave / Convexe

The higher collagen Type II synthetic levels and increased total protein content with no matrix turnover suggest that scoliotic changes are due to an altered and ineffective response to a pathologic mechanical environment.
Inter vertebral disc  IVD

APOPTOSE

70 Discs from 16 Scoliotic patients (8 M – 8 F) 9 AIS, 7 Neuro Musc.
Mean age : 20.6 Y (10 – 48 Y)

Increased number of apoptotic cells in the nucleus of scoliotic discs and the apex disc suppose a pivotal role of programmed cell death for the progression of this common disorder.

The simultaneous increase of Fas and Fas - ligand expression in areas with increased cell death point to an activation of the apoptotic process via the Fas/Fas-L system.

A mathematical model was used to determine the contribution of collagen fibre orientation and directional imbalance within the annulus fibrosus to vertebral rotation and overall deformity of thoracic spine.

Imbalance in the ratio of clockwise and anti-clockwise collagen fibres within the annulus fibrosus has been demonstrated to have the potential to contribute to the progression of scoliosis.
Morphology

Professor Alain Berthoz, Collège de France
• Observation of cranio-facial asymmetry: CT of the skull

Professor Robert Pashman, Cedar Sinai Institute for spinal disorder, Los Angeles, USA
• Circulation of the cerebro-spinal fluid: convexe side compare to concave, apex of the scoliosis
Morphology

Height and AIS

Taller mean standing height in girls with scoliosis compared to healthy control

Girls with AIS have an earlier growth spurt and earlier attainment of adult height

Without correction for the error due to scoliosis itself

| Length measurements of the sitting height | Corrected using the method described by Bjure and Al. |

No difference in growth pattern or height between AIS and control group
Paravertebral Musculature

Muscle's abnormalities

Muscle spindle
Individual muscle fiber morphology
Histochemistry
Electromyography
Sarcolemma abnormalities at the muscle - tendon junction
Calcium, copper and zinc concentrations
Muscle imbalance
Calmodulin

Platelet = « mini » skeletal muscle

- Protein contractile system: Actin and Myosin
- No axial attachments
- Calmodulin regulates the contractile properties through its interaction with actin and myosin

Secretion in correlation with melatonin

Dr Jocelyne Enouf, Hôpital Lariboisière, Paris.
Results. Calmodulin levels increased in all the patients with progressive curves (13/13), remained stable in 73% of the patients with nonprogressive curves (11/15), and were higher generally in curves greater than 30° and double structural curves. Calmodulin levels usually decreased in patients undergoing brace treatment (14/17) or spine fusion (9/10).

Correlation with nonprogressive curves was not as consistent, with 27% non correlation.

Biomechanical spine finite element model

Five pathogenesis hypotheses of AIS from an initial geometrical eccentricity:

**Line imbalance of 3mm or 2° rotation leads to right thoracic scoliosis up to 39° with an apical vertebral rotation up to 7° while the apical wedging increase to 8.5°**

A sole eccentricity in the sagittal plane generated a non significant frontal plane deformity

Deacon, Dickson and co-workers reported that the height of the anterior vertebral body of the apical vertebra was significantly greater than the posterior vertebral body height.

Thoracic lordosis, which is caused by an overgrowth of the anterior part of the vertebral body, triggers the initiation of scoliosis by buckling.
Backward inclination of the thoracic vertebrae rather than lordotic condition could play a role in the mechanism of rotation and thus the onset and progression of AIS.

Facet orientation, predominant posterior location and longitudinal alignment of major muscles and ligament are not well suited to counteract dorsal shear forces.

Rib osteotomy/shortening in growing rabbits induces scoliosis

In 5 of 6 deceased women with right convex thoracic IS, the left ribs were somewhat longer than the right ones.

The left breast in scoliotic, but not in normal, girls was significantly more vascularized than that of the right one.

In growing rabbits resection of 4 intercostal nerves, including the sympathetic fibres, leads to increased vascularity of the denervated hemithorax, increased osteogenetic activity at the costochondral junction, increased longitudinal rib growth and progressive scoliosis concave to the side of denervation, with decreased kyphosis and vertebral rotation to the convexity.

The Thoracospinal Theory

Forced mechanical lengthening of one rib in rabbits produces scoliosis

The thoracospinal theory of the pathogenesis of IS according to which overgrowth of the left ribs, due to hypervascularity of the ipsilateral anterior hemithorax, alters the equilibrium of forces controlling the alignment of the normal spine, as it is predisposed to rotate to the right, and triggers the thoracospinal deformity in IS.

The theory better than any other hypothesis explains the mode of origin of at least the most common form of IS, with location on the thoracic spine, concavity to the left, apex at the T7-T9 level, vertebral rotation to the right, decreased kyphosis and almost exclusive affection of adolescent girls.

Postural Control

Body's internal representation

Muscular effector

Visual inputs

Sensory strategies

Vestibular inputs

Somesthesic inputs
Equilibrium and posture

Docteur Sylvette Wiener-Vacher, Paris
• Predictive and prognosis of vestibular and otolithic tests

Professor Jack Chun Yiu Cheng, Chinese University of Hong Kong
• Relationship between postural equilibrium, evoked somato-sensitive potential and progression of the curve
Equilibrium and posture

Defect in the Vestibular System

Semicircular canal examination

Asymmetric sensitivity


Postural Control

statokinesiograph

Postural Control

Oscillation amplitude 4°
frequency 0.5 Hz

TYPE 1
slow dynamic test
### Postural Control

#### Posturography

<table>
<thead>
<tr>
<th>Conditions</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vision</td>
<td>+</td>
<td>-</td>
<td>Faussée</td>
<td>+</td>
<td>-</td>
<td>Faussée</td>
</tr>
<tr>
<td>Somesthésie</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Faussée</td>
<td>Faussée</td>
<td>Faussée</td>
</tr>
<tr>
<td>vestibule</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Postural Control

Equilibrium Score

FALL
1 2 3 4 5 6
Conditions

Composite
80
Postural Control

Slow sinusoidal dynamic tests
In group IS >13° (Cobb angle):
• Greater preference in visual inputs
• Greater instability and postural adjustment = lower integration of informations
• Relationship between the importance of spine deformation and the importance of postural control disorders for IS not very severe at the beginning of their evolution.
Postural Control

• These sensitive and powerful tools allow the search for a predictive index of the progression of a scoliosis.

The posturography would represent a non invasive examination of choice to pose the indication of an orthopaedic, surgical treatment even medicinal in the future.
Equilibrium and posture

Disorders in smooth visual pursuit in AIS

Disorders in vestibular – induced nystagmus in AIS


Proprioceptive disorders in AIS

**Equilibrium and posture**

**Disturbed vestibulo-ocular reflex in AIS**


**Spontaneous occurrence of nystagmus in AIS**


**Gravity-sensitive otolith examination discloses a correlation between idiopathic scoliosis and vestibular asymmetry.**

Postural Control

Sensory feedback from several spinal cord levels on both sides of the spinal cord. This sensory information also is transferred to higher levels in the brain, including the nucleus gracilis and cuneatus, the vestibular nuclei, and the thalamus.

The presence of Fos indicates neurons that had been stimulated.

Mechanical stretch on the intertransverse ligament on the right side at T3 - T4 in 4 week-old-chickens.

### Postural Control; Vision

<table>
<thead>
<tr>
<th>Children visually handicapped</th>
<th>Healthy control participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mean age: 11 Y 7 m) 47 M 28 F</td>
<td>(mean age: 10 Y 2 m) 464 M 264 F</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical exam.</th>
<th>Moire</th>
<th>RX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rib hump &gt; 5mm</td>
<td>At least 1 fringe</td>
<td>Rot &gt; 5° Cobb &gt; 10°</td>
</tr>
</tbody>
</table>

| 75 Visually handicapped | 26 (34.7%) | 18 |
| 728 control | 42 (5.7%) |   |

1/3 Cervico-thoracic curves 3/26 evolving conditions

Melatonin and vision

60 Chickens divided in three groups:

<table>
<thead>
<tr>
<th>Group</th>
<th>Scoliosis</th>
<th>Right</th>
<th>Left</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (20) = Pinealectomy</td>
<td>65%</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>Group 2 (20) = Pinealectomy + left side blinded</td>
<td>55%</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Group 3 (20) = Pinealectomy + Right side blinded</td>
<td>60%</td>
<td>7</td>
<td>6</td>
</tr>
</tbody>
</table>

Visually impaired chickens have a significantly higher likelihood of left thoracic curves regardless of the side of the blindness.