Idiopathic scoliosis

research supported by

INSTITUT DE FRANCE

FONDATION YVES COTREL

POUR LA RECHERCHE EN PATHOLOGIE RACHIDIENNE

2000-2015

Boldness and effort sharing

The creation of a foundation is very often rooted in a humanitarian reflection. The Cotrel Foundation-Institut de France, created in 1999, does not deviate from this rule.
It is the continuation Doctor Yves Cotrel's personal journey. His insatiable curiosity and openness of spirit, and if I dare say, openness of heart, fueled his quest to improve the fate of others.

The Cotrel Foundation has been able to take on the qualities of boldness and collaboration, from a man who is preoccupied by the well-being of those who suffer.

Today, the foundation brings together more than a hundred researchers from all nationalities and disciplines, moved by the same perseverance in its service to help the sick throughout the world.

This family foundation has naturally found its place in the heart of the Institut de France where it can fulfill its philanthropic mission. It is a successful synthesis of longstanding human values and modern scientific rigor.

The Institut de France is proud to accompany and support their missions

Gabriel de Broglie
Chancellor of the Institut de France

A mission is not one that you take, but one you are given

The foundation for research in spinal pathological was born on a simple assessment : my professional life was punctuated by encounters and occurrences that traced a winding path on which I always felt guided and lead.

The commitment to the service of the sick naturally followed on into research. With the backing of my family, and with the hope of mobilizing, stimulating and assembling knowledge and willingness, we started the foundation. Its first research program was an ambitious challenge of identifying the causes and mechanisms in the evolution of idiopathic scoliosis.

The worry I once saw in the eyes of the patients and the inability to answer their questions, sometimes unspoken, asking again without any answer, "Scoliosis? Why? How? " remained.

This mission, whose concern for the human person is inseparable from the scientific fundamentals, has found a haven at the Institut de France whose respect for rigor and values are ours.

I would like to thank the patients who educated me and all who today have joined me on this road that I hope will continue.

Docteur Yves Cotrel
Founder

Why support the Foundation ?

Although early screening and surgical treatments have considerably progressed over the past few years, we must admit that these are only palliative treatments. The real problem lies elsewhere.
We must go back to the source

The Foundation has decided to select and finance the best research programs focusing on the origin of idiopathic scoliosis in order to provide early detection of its progressive forms, and even better, to prevent its occurrence.

The questions raised fifty years ago are still waiting to be answered today.

Yves Cotrel

For your donations :
Caisse des dépôts et consignations
in Favour of : Fondation Yves Cotrel - Institut de France
Compte : 40031 00001 0000299249V 95
IBAN : FR62 4003 1000 0100 0029 9249 V95
BIC CDCG FR PP

Each gift is fully dedicated to research, with no administrative cost
Check your tax deductibility according to your country of origin

To apply for a grant :

The Scientific Board will study all applications submitted by March 31st of each year
The research theme is: Etiology of idiopathic scoliosis
Check the online registration form at www.fondationcotrel.org

For further questions, please contact us: mullerconte@fondationcotrel.org

The Scientific Board

It is composed of 11 members who select which projects will be supported each year.
Responsible for upholding ethics, the council assures a regular monitoring of each team: to their commitment to circulate information and to participate in the assembly organized once a year at the Institut de France in Paris.

President: **Dr. Yves Cotrel**

Pr. Jean Dubousset
Emeritus Professor of university (René Descartes)
Member of Académie Nationale de Médecine and Académie de Chirurgie

Dr. Michel Guillaumat
President of Société Française de Chirurgie Orthopédique et Traumatologique (SoFCOT) 1997-98

Pr. Pierre Hardouin
Professeur à l'Université du Littoral Côte d'Opale, Nord-Pas-deCalais

Pr. Stuart Edelstein
Member of Académie des Sciences
Professor at Ecole Normale Supérieure
Research associate, University College, Londres

Pr. François Gros
Honorary Perpetual secretary of Académie des Sciences
Biologist
Honorary professor, Collège de France

Professeur Michel Fardeau
Member *correspondant* of Académie des Sciences
Emeritus research director, CNRS
Myology institute

Dr. Nicolas Plais-Cotrel
Orthopaedic surgeon

Pr. Margaret Buckingham
Member of Académie des Sciences
Professor at Institut Pasteur
Molecular biologist

Dr. Marie-Hélène Plais-Cotrel

Pr. Norbert Pasutti
Orthopaedic surgeon
President of SoFCOT 2014

The white paper of idiopathic scoliosis provides a record of recent results and in particular the main disturbances associated with scoliosis that are studied by scientific teams financed by the **Fondation Yves Cotrel – Institut de France.**

Scoliosis is a three dimensional deformation of the vertebral column that occurs and develops during growth. In 3 out of 4 cases, its origin is unknown (*idiopathic scoliosis*) and its evolution is variable often responding favorably to simple treatments, but sometimes more severe and necessitating surgical intervention.

The problematic is twofold.
On one hand, detecting progressive forms as early as possible allows for treatment to be most effective. On the other hand, research in the cause of scoliosis allows for action upon the mechanisms responsible for these deformations and not only on their consequences.

The Fondation Yves Cotrel – Institut de France provides a consolidation of information to aid in improving early detection of scoliosis. It finances research work to discover the cause of scoliosis. Over the last 15 years, 52 teams from 9 countries have been financed for a total over of 3,7 millions euros.

Scoliosis reveals itself today as a complex disease with roots in several mechanisms. Understanding these abnormalities is crucial. It could lead to perfecting early detection of evolutive scoliosis or in terms of prevention, paving the way in bringing hope to avoid heavy surgical treatments.

In order to understand the cause of scoliosis, multiple research avenues have been pursued such as spinal biomechanics, cellular biology and genetics. These studies have allowed discoveries or classifications of the diverse perturbations associated with scoliosis. While some of them are possible consequences of the deformation itself, others are probably connected to the genesis of the scoliosis.

The separation into chapters is designed to aid in reviewing the findings. As the researches often broach a wide range of aspects, this classification is not exclusive. However, **it illustrates the complexity idiopathic scoliosis** and we hope it may entice new teams to apply for grants.

We hope, that this presentation provides you with a general idea of the most recent work in scoliosis and of the important progress obtained over the last few years, with the financial support of the Fondation Yves Cotrel - Institut de France and entices the reader to submit new projects.

Table of contents

CHAPTER I

Biomechanical Investigation

Scoliosis is a major deformation of the Spine and the biomechanical studies aim at describing the development of the normal and the scoliosis spine, understanding the imbalances that arise during growth and analyzing the alterations which appear at the disk levels and the vertebral bodies.

Scoliosis can be studied by analyzing the rachidian curvatures and the constraints on the vertebral column, but also by examining the modifications of the intervertebral disks.

New technologies as The EOS Imaging system and 3D reconstruction of the Spine allow a modelization of the deformation and the development of new tools that assess the risk of evolution of the deformation

The role of intrinsic spinal mechanisms in etiopathogenesis of AIS

The Utrecht scoliosis team consists of the orthopedic surgeons **René Castelein** and Moyo Kruijt, imaging engineers Koen Vincken and Wilbert Bartels, and (former) MD/PhD students Jan-Willem Kouwenhoven, Michiel Janssen, Tom Schlösser, Dino Colo and Rob Brink.

Our scoliosis research line has focused on the biomechanical characteristics of the spine, both in patients with a deformity as well as those without a deformity or at risk for developing one. Several studies have been done in a joint collaboration between our group at the Department of Orthopedic Surgery and the Image Sciences Institute Utrecht. In 2005, a new and original hypothesis on the role of posteriorly directed shear loads in the aetio-pathogenesis of idiopathic scoliosis was put forward.[1] Since then, new collaborations were started with the Chinese University of Hong Kong, Shatin, Hong Kong (professor Jack C. Cheng, MD, FRCSEd), the Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA (John P. Dormans, MD, former chief and John M. Flynn, MD, current chief) and Nemours/Alfred I. duPont Hospital for Children, Wilmington, Delaware, USA (Suken A. Shah, MD).

So far, it has been shown that: (1) these posteriorly directed shear loads act only on the human erect spine[2-4], (2) these loads decrease the rotational stability of the spine *in vitro* and *in vivo*[6-8] and (3) a longer area of the female spine is more subject to posteriorly directed shear loads as certain areas in the female spine are more posteriorly inclined, especially during the adolescent growth spurt (Figure 1A).[3,5]

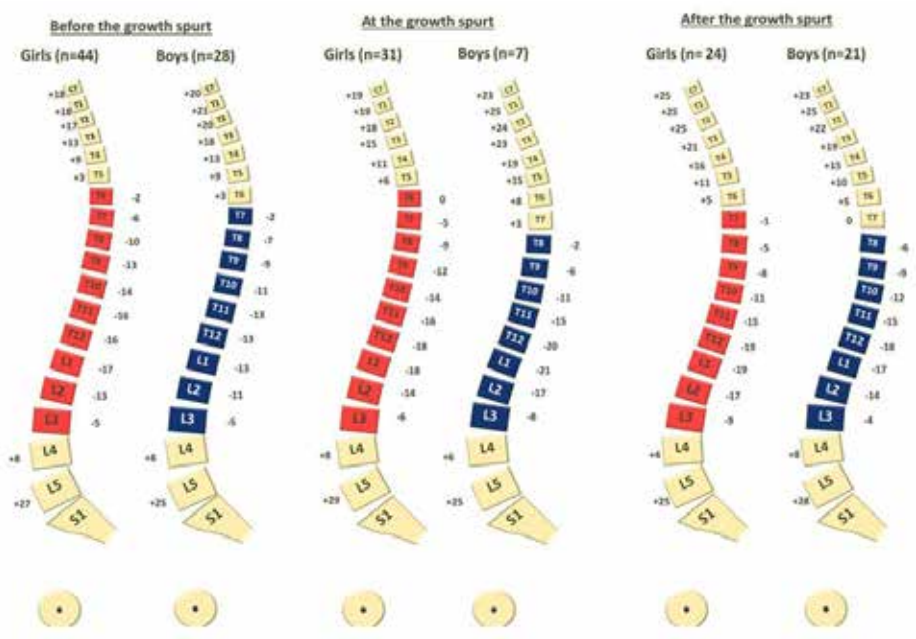


Figure 1A. Girls, especially at the peak of their growth spurt, have a longer posteriorly inclined segment (red) than boys at the same stage of development (data compiled from Schlösser et al.[5]).

Thoracic kyphosis is also less pronounced at the peak of the growth spurt in girls, as compared to boys (Figure 1B).[5] Furthermore, we showed that once rotation occurs in a developing scoliosis, it logically follows an already built-in vertebral rotational pattern, which is pre-existent in the normal paediatric[9,10] and adult human spine[11-13].This rotation matches the asymmetrical closure of the neurocentral junctions in the growing spine[10] and is related to organ anatomy, but not to handedness[13]. Furthermore, in a recent research project involving

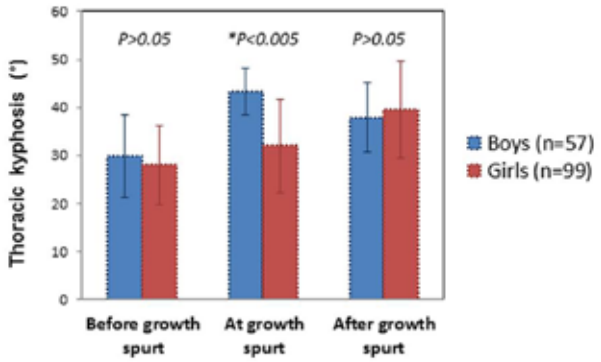


Figure 1B. Girls at the peak of their growth spurt have less thoracic kyphosis than boys at the same stage of development (data compiled from Schlösser et al.[5]).

scoliotic children with mild curves, a difference in the sagittal profile of the spine was reported between different types of idiopathic scoliosis; a significantly decreased kyphosis and a more cranial posteriorly inclined segment in thoracic scoliosis compared to a more caudal similar segment in lumbar idiopathic scoliosis (Figure 2). This again demonstrates that the posteriorly inclined vertebrae take part in the development of different curves, and further confirms the above described aetio-pathogenetic theory.[14,15] More recently, we reported about the true three-dimensional (3-D) morphology of idiopathic scoliosis and described that all curves in scoliosis, primary as well as secondary ones, demonstrate an apical lordosis (which has been described before) but that the junctional zones are straight.[16] Anterior overgrowth, as it has been termed, is thus predominantly present in the apical zones of each curve, and is not a global disturbance of growth of the spine. Most of the deformity in all planes (e.g. torsion, as well as coronal and sagittal wedging) is located in the disc as compared to the vertebral body. The anterior side was observed to be 8% longer across the apex on average. Beside elucidating the true nature and morphology of the disorder it has also implications for surgical strategy. In order to restore the sagittal balance as optimal as possible, posterior lengthening or anterior shortening should be performed.[16]

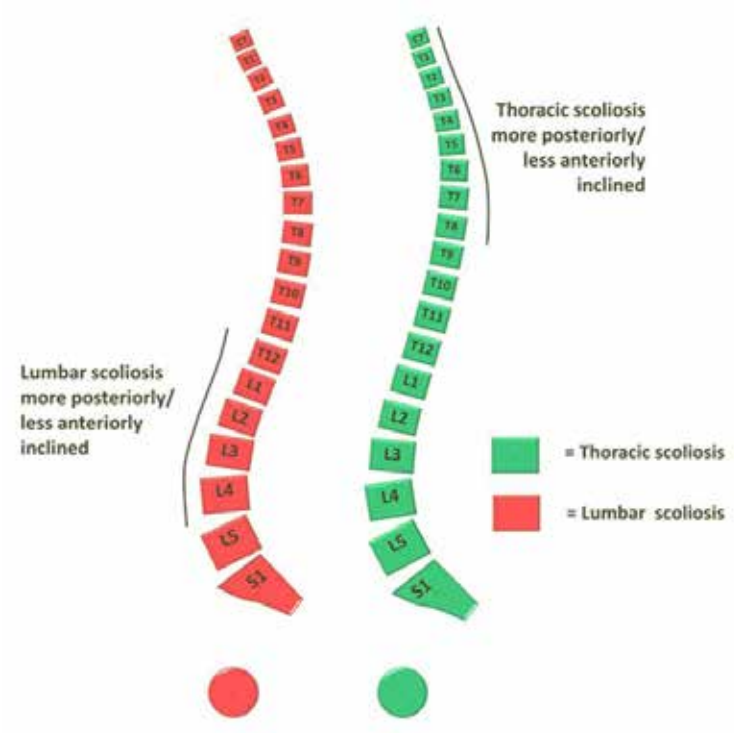


Figure 2. The sagittal profile of the spine in mild thoracic scoliosis shows a longer and more proximal area of posterior inclination than the sagittal profile in mild lumbar scoliosis (data compiled from Schlösser et al.[14]).

References

1. Castelein RM, van Dieen JH, Smit TH. The role of dorsal shear forces in the pathogenesis of adolescent idiopathic scoliosis—a hypothesis. Med Hypotheses. 2005;65:501-508.

2. Janssen MM, de Wilde RF, Kouwenhoven JW, et al. Experimental animal models in scoliosis research: A review of the literature. Spine J. 2011;11:347-358.

3. Janssen MM, Drevelle X, Humbert L, et al. Differences in male and female spino-pelvic alignment in asymptomatic young adults: A three-dimensional analysis using upright low-dose digital biplanar X-rays. Spine (Phila Pa 1976). 2009;34:E826-32.

4. Janssen MM, Kouwenhoven JW, Castelein RM. The role of posteriorly directed shear loads acting on a pre-rotated growing spine: A hypothesis on the pathogenesis of idiopathic scoliosis. Stud Health Technol Inform. 2010;158:112-117.

5. Schlösser TP, Vincken KL, Rogers K, Castelein RM, Shah SA. Natural sagittal spino-pelvic alignment in boys and girls before, at and after the adolescent growth spurt. Eur Spine J. 2015 Jun;24(6):1158-67.

6. Kouwenhoven JW, Castelein RM. The pathogenesis of adolescent idiopathic scoliosis: Review of the literature. Spine (Phila Pa 1976). 2008;33:2898-2908.

7. Kouwenhoven JW, Smit TH, van der Veen AJ, Kingma I, van Dieën JH, Castelein RM. Effects of dorsal versus ventral shear loads on the rotational stability of the thoracic spine: a biomechanical porcine and human cadaveric study. Spine (Phila Pa 1976). 2007 Nov 1;32(23):2545-50.

8. Janssen MM, Vincken KL, Kemp B, et al. Pre-existent vertebral rotation in the human spine is influenced by body position. Eur Spine J. 2010;19:1728-1734.

9. Janssen MM, Kouwenhoven JW, Schlösser TP, et al. Analysis of preexistent vertebral rotation in the normal infantile, juvenile, and adolescent spine. Spine (Phila Pa 1976). 2011;36:E486-91.

10. Schlösser TP, Vincken KL, Attrach H, Kuijf HJ, Viergever MA, Janssen MM, Castelein RM. Quantitative analysis of the closure pattern of the neurocentral junction as related to preexistent rotation in the normal immature spine. Spine J. 2013 Jul;13(7):756-63.

11. Kouwenhoven JWM, Vincken KL, Bartels LW, Castelein RM. Analysis of Preexistent Vertebral Rotation in the Normal Spine. Spine 2006;31(13):1467-72.

12. Kouwenhoven JWM, Vincken KL, Bartels LW, Meij BP, Öner FC, Castelein RM. Analysis of Preexistent Vertebral Rotation in the Normal Quadruped Spine. Spine 2006;31(20): E754-58.

13. Kouwenhoven JWM, Bartels LW, Vincken KL, Viergever MA, Verbout AJ, Delhaas T, Castelein RM. The Relation Between Organ Anatomy and Preexistent Vertebral Rotation in the Normal Spine – Magnetic Resonance Imaging Study in Humans With Situs Inversus Totalis. Spine 2007;32(10):1123-28.

14. Schlösser TP, Shah SA, Reichard SJ, Rogers K, Vincken KL, Castelein RM. Differences in early sagittal plane alignment between thoracic and lumbar adolescent idiopathic scoliosis. Spine J. 2014 Feb 1;14(2):282-90.

15. Cheng JC, Castelein RM, Chu WC, Danielsson AJ, Dobbs MB, Grivas TB, Gurnett CA, Luk KD, Moreau A, Newton PO, Stokes IA, Weinstein SL, Burwell RG. Adolescent idiopathic scoliosis. Nat. Rev. Dis. E-Pub, 2015 Sept 24.

16. Schlösser TP, van Stralen M, Brink RC, Chu WC, Lam TP, Vincken KL, Castelein RM, Cheng JC. Three-Dimensional Characterization of Torsion and Asymmetry of the Intervertebral Discs versus Vertebral Bodies in Adolescent Idiopathic Scoliosis. Spine (Phila Pa 1976). 2014 Jun 11.

Additional publications from this research group

Schlösser TPC, Colo D, Castelein RM. Etiology and Pathogenesis of Adolescent Idiopathic Scoliosis. *Sem Spine Surg*, 2015Mar 27 (1) 2-8.

Kouwenhoven JW, Janssen MM, Castelein RM. Letter to the editor concerning: Z.D. Yang et al., There may be a same mechanism of the left-right handedness and left-right convex curve pattern of adolescent idiopathic scoliosis, Med. Hypotheses (2010) Oct 29. Med Hypotheses. 2011 Jul;77(1):156.

Janssen MM, Vincken KL, van Raak SM, Vrtovec T, Kemp B, Viergever MA, Bartels LW, Castelein RM. Sagittal spinal profile and spinopelvic balance in parents of scoliotic children. Spine J. 2013 Dec;13(12):1789-800.

Schlösser TP, Stadhouder A, Schimmel JJ, Lehr AM, van der Heijden GJ, Castelein RM. Reliability and validity of the adapted Dutch version of the revised Scoliosis Research Society 22-item questionnaire. Spine J. 2014 Aug 1;14(8):1663-72.

Schlösser TP, Janssen MM, Vrtovec T, Pernuš F, Oner FC, Viergever MA, Vincken KL, Castelein RM. Evolution of the ischio-iliac lordosis during natural growth and its relation with the pelvic incidence. Eur Spine J. 2014 Jul;23(7):1433-41.

Castelein RM. The benefit of braces in adolescent idiopathic scoliosis. Ned Tijdschr Geneeskd. 2014;158(4):A7246. Dutch.

Schlösser TP, van der Heijden GJ, Versteeg AL, Castelein RM. How 'idiopathic' is adolescent idiopathic scoliosis? A systematic review on associated abnormalities. PLoS One. 2014 May 12;9(5):e97461.

Homminga J, Lehr AM, Meijer GJ, Janssen MM, Schlösser TP, Verkerke GJ, Castelein RM. Posteriorly directed shear loads and disc degeneration affect the torsional stiffness of spinal motion segments: a biomechanical modeling study. Spine (Phila Pa 1976). 2013 Oct 1;38(21):E1313-9.

Vrtovec T, Janssen MM, Likar B, Castelein RM, Viergever MA, Pernuš F. A review of methods for evaluating the quantitative parameters of sagittal pelvic alignment. Spine J. 2012 May;12(5):433-46.

Vrtovec T, Janssen MM, Likar B, Castelein RM, Viergever MA, Pernuš F. Evaluation of pelvic morphology in the sagittal plane. Spine J. 2013 Nov;13(11):1500-9.

Vrtovec T, Janssen MM, Pernuš F, Castelein RM, Viergever MA. Analysis of pelvic incidence from 3-dimensional images of a normal population. Spine (Phila Pa 1976). 2012 Apr 15;37(8):E479-85

Kouwenhoven JW, Van Ommeren PM, Pruijs HE, Castelein RM. Spinal decompensation in neuromuscular disease. Spine (Phila Pa 1976). 2006 Apr 1;31(7):E188-91.

Holewijn R, Schlösser TP, van der Veen AJ, Bisschop A, Smit T, Castelein RM, de Kleuver M. How much does a Ponte osteotomy contribute to spinal flexibility in scoliosis surgery? Spine Deformity. 2015 Sep.

PhD-theses, principal investigator René M. Castelein, MD PhD

Jan-Willem Kouwenhoven. The Role of Intrinsic Spinal Mechanisms in the Pathogenesis of Adolescent Idiopathic Scoliosis. Utrecht University, 2007.

Michiel M.A. Janssen. Human Upright Spinopelvic Alignment and the Etio-Pathogenesis of

Idiopathic Scoliosis. Utrecht University, 2011.

Tom P.C. Schlösser. Adolescent Idiopathic Scoliosis: from Normal Spinal Anatomy to Three-Dimensional Deformity. Utrecht University, 2014.

Changes in the intervertebral disc in development of the scoliotic deformity

The work described has been carried out by **Professor Jeremy Fairbank,** consultant orthopaedic surgeon,
Dr Jing Yu and Dr Jill Urban, research scientists
University of Oxford,
United Kingdom

Deformation of the intervertebral disc plays a major role in the scoliotic deformity. Its wedging is thought to be secondary to altered loading.
The abnormal mechanical stresses which arise during the development of the deformity, could alter cellular activity and hence lead to abnormalities of the extracellular matrix and to matrix calcification. The pressures arising in the disc are unknown and little is known concerning the effect of the scoliotic deformity on disc cells and on the extracellular matrix which regulates disc biomechanical behaviour.

Hypothesis and Methodology

Our hypothesis is that scoliotic discs are under abnormal pressures and that these affect cellular function and hence composition of the disc extracellular matrix, leading to a further disc deformation, as described by the Heuter-Volckmann effect.

We examined the pressure across scoliotic intervertebral discs by inserting a calibrated pressure transducer into the discs during routine surgical procedures. We examined cells and the elastic network of tissue removed during surgery, using immunohistochemistry. To further examine the role of the elastic network, we followed changes in the spine of mice with abnormalities of the elastic network during growth (Tsk mice) and compared results with wild type-mice.

Results:

a) Pressure measurements

We found that stresses in scoliotic discs are abnormal. Scoliotic discs in recumbent anaesthetised, muscle relaxed patients have higher nuclear hydrostatic pressures than those measured in non-scoliotic discs (Figure 1).

The results indicate remodeling of tissues occurs under asymmetrical loading leading to stiffening of the disc and suggesting changes took place in the structural framework of the disc.

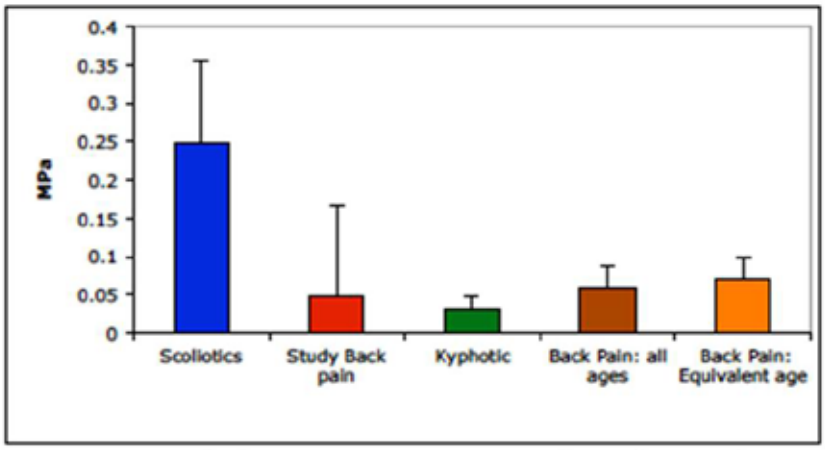


Figure 1. Mean hydrostatic pressures measured in scoliotic and non-scoliotic discs during surgical procedures [1].

b) Changes in elastin and collagen networks in scoliotic discs

Histological examination of discs from adolescent spines showed that normal discs (from trauma patients) had an abundant well organised elastin network in the annulus fibrosus of the disc. In the scoliotic disc however, the collagen lamellae were disorganised as was the sparse elastic fibre network (Figure 2).

These differences in structural networks between normal and AIS discs would be expected to affect spine biomechanical function and also cellular function through changes in growth factor binding on loss of elastic fibre density.

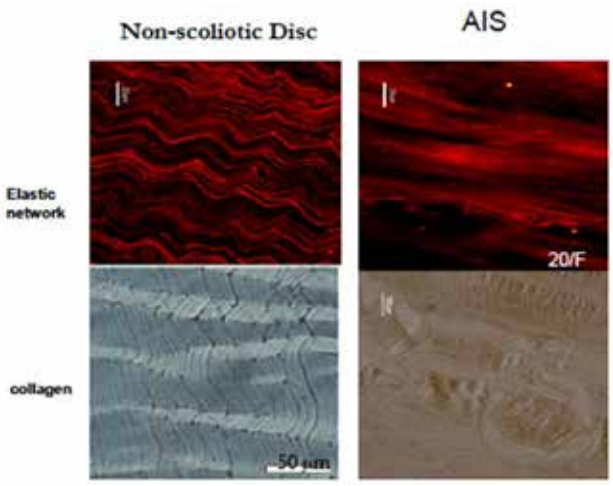


Figure 2. The well organised collagen and elastic networks in a normal disc and the sparse and disorganised networks in an AIS disc.

c) Transgenic mice

We examined the differences in mice with a defect in growth factor binding similar to that induced by elastic fibre abnormalities (TSK mice), relative to growth in normal mice (WT mice). Up till the age of 4 weeks, the TSK mice grew more slowly than the WT. Between 6-10 weeks
the transgenics grew very rapidly and developed a much greater kyphosis and Cobb angle than controls and their tendons were much stiffer (not shown).

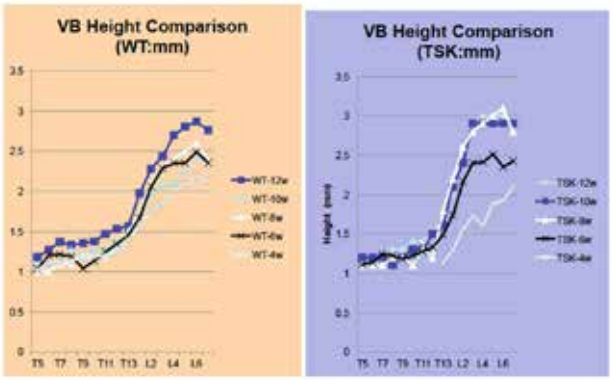


Figure 3. Vertebral height changes with age and spinal level in WT and TSK mice[3]

Conclusions

The role of the elastic network in development of AIS has long been of interest and abnormalities in cells and matrix of AIS patients have been shown previously and also in studies reported here. Recent genetic studies and also the effect of abnormalities related to the elastic network on growth of mice further point to its importance in at least a subset of AIS patients.

References

1. Modi HN et al Differential wedging of vertebral body and intervertebral disc in thoracic and lumbar spine in adolescent idiopathic scoliosis - A cross sectional study in 150 patients. Scoliosis. 2008;3:11.

2. Villemure I, Stokes IAF. Growth plate mechanics and mechanobiology. A survey of present understanding. J Biomechanics. 2009 Aug 25;42(12):1793–803.

3. Roberts S, et al The cartilage end-plate and intervertebral disc in scoliosis: calcification and other sequelae. J Orthop Res. 1993 Sep;11(5):747–57

a. Aigner T et al Variation with age in the pattern of type X collagen expression in normal and scoliotic human intervertebral discs. Calcified Tissue International. 1998 Sep;63(3):263–8..

Papers arising

1. Meir AR, Fairbank JCT, Jones DA, McNally DS, Urban JPG. High pressures and asymmetrical stresses in the scoliotic disc in the absence of muscle loading. Scoliosis. 2007;2:4.

2. Internal pressure and stress environment of the scoliotic intervertebral disc—a review. Proc Inst Mech Eng H 2008 Feb;222(2):209–19.

3. Bibby, SR, Meir A, Fairbank JCT, Urban, JPG. Cell viability and the physical environment in the scoliotic intervertebral disc. Stud Health Technol 2002;91:419–21.

4. Yu J, Fairbank JCT, Roberts S, Urban JPG. The elastic fiber network of the anulus fibrosus of the normal and scoliotic human intervertebral disc. Spine. 2005 Aug 15;30(16):1815–20.

5. Yu J, Tirlapur U, Fairbank J, Handford P, Roberts S, Winlove CP, et al. Microfibrils, elastin fibres and collagen fibres in the human intervertebral disc and bovine tail disc. J Anat. 2007 Apr;210(4):460–71.

6. Li B, Urban JPG, Yu J. The distribution of fibrillin-2 and LTBP-2, and their co-localisation with fibrillin-1 in adult bovine tail disc. J Anat. 2012 Feb;220(2):164–72.

The growth of the scoliotic spine: an unstable dynamical system ?

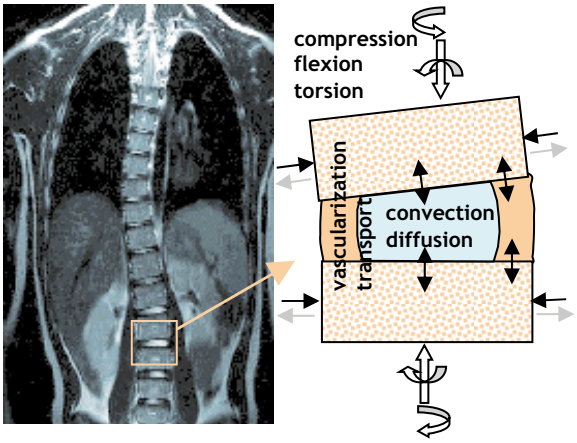
Our collaborative research project has been built with four public research and clinical team partners having complementary scientific and technical skills. The **IMFT** CNRS in Toulouse (https://www.imft.fr) is the largest French laboratory in fluid mechanics. For the study of transport phenomena in living tissues, the "Porous Media Study Group" has developed close and long-term collaborations with well-established fife sciences teams (biologists, clinicians, healthcare companies, here **Pascal Swider**).

The **Children Hospital at Toulouse** and IMFT have been collaborating for more than ten years on studies dealing with idiopathic scoliosis. This group initiated approaches in biological tissue poromechanics to investigate the experimental and numerical modelling of intervertebral discs. The integration of these facilities in a single place (Toulouse University Hospital) is a key point for the success of our project.

The **LMGC** CNRS in Montpellier (http://www.lmgc.univ-montp2.fr) has a significant experience in theoretical and numerical multi-physics modelling of coupled phenomena, particularly regarding to transport mechanisms in deformable porous media.

Background :

The spine growth is governed by underlying mechano-biological phenomena. In scoliosis, it is characterized by pronounced ephebic growth and retardation in the bone maturation of vertebral bodies. Scoliotic segments subjected to compression, flexion and torsion stresses underlies mechanical loading beyond normality. The resulting strain energy is mainly located into the intervertebral disc and to a lesser extent in the vertebral end plates and facets. It is observed that the segment homoeostasis is controlled by nutrient exchanges that mainly occur through vertebral end plates (Bibby et al. 2005) and that scoliotic discs show modified hydration levels (Gu et al. 1998; Abelin-Genevois et al. 2015)



Hypothesis and Methodology

The central hypothesis is to assume the adolescent scoliotic spine as a non-linear dynamical system characterized by evolution instabilities. As a corollary, it is considered that this non-equilibrium phenomenon in time and space might be caused by pathological tissue remodelling. Instabilities might appear when an initial disturbance grows during the evolution of the system. This initial disturbance can result from various sources. Depending on external conditions, the consequences are damped or amplified in a pathological manner. These instabilities are induced by a series of physiological, biochemical and mechanical episodes. It can lead to an amplification loop of scoliosis curvatures in flexion-torsion.

To study this phenomenon, we use mechanobiological disturbances accessible from clinical settings: spine deformation (X-ray database), hydration level of segment components (MRI database), strain energies and mechanical loadings (numerical models database).

- Key- role of mechanical loads and nutrients exchanges: how is the homeostasis of the spinal segment modified by the alterations of local poromechanical properties?

- Implication of mechano-bio-chemical factors in the growth processes of the vertebral segment: what is the influence of initial conditions?

- Instability of regulation loops: Is the spine curvature progressive or stable?

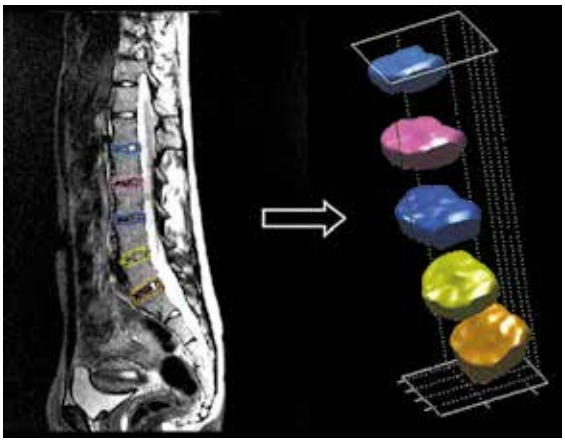
Results

In the **control group**, disc volumes and hydration content followed the lumbar lordosis pattern. Highest values were observed at the apical lordotic levels whereas junctional discs demonstrated lower disc volumes. Volume variability was higher in junctional levels (50 %) and inter individuals variations ranged about 30 %**.** Variations correlated with age with increasing values of disc and nucleus volumes. Volumes increased during the adolescent growth spurt (lordosis, pelvic morphology and sagittal alignment, evolution of mechanical stimuli with growth). Hydration ratio remained stable for all lumbar discs (28 %).

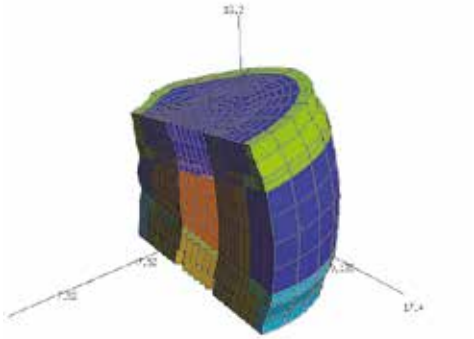
In the **scoliosis group**, disc volumes were slightly reduced compared with control and hydration matched with the lowest value observed in normal subjects and did not vary according to the lumbar level. In operated patients, disc volumes remained stable while nucleus pulposus volumes showed a tendency to increase.

In longer-term post-op (6 years), disc volumes slightly decreased and hydration increased, the proximal part of the lumbar spine became «overhydrated» compared to controls**.**

We developed **theoretical and numerical methodologies** to investigate the response of spine segments. It involved poro (hyper) elasticity modelling and reactive behaviour (Ambard et al. 2009). Convective and diffusive properties of tissue played a significant role in the segment response. The scoliotic curvature showed a limited influence on the amount of exchanged masses whereas a migration of fluid flux centre towards thicker zone of discs was predicted. In scoliosis, the coupling between bending in the frontal plane and the torsion as well predicted as observed in clinical setting. No return to equilibrium might be a consequence of stress increase and alteration in mass transports and tissue growth and remodelling.



Disc 3D reconstruction from MRI



Conclusion

The originality of our approach consisted in a) establishing an interdisciplinary strategy driven by the clinical setting (unique children database) and reinforced by advanced numerical methodologies in mechanobiology. Results from our clinical database and numerical models tended to confirm our central hypothesis. We are currently working on identifying dynamical instability criteria to help in the early diagnosis and surgical treatment (if necessary). This will improve the comfort and the follow-up of patients.

References:

Gu et al. (1998) A mixture theory for charged-hydrated soft tissues containing multi-electrolytes: passive transport and swelling behaviors. J. Biomech. Eng., 120: 169–180.

Bibby et al. 2005. Metabolism of the intervertebral disc: effects of low levels of oxygen, glucose, and pH on rates of energy metabolism of bovine nucleus pulposus cells. Spine, 30: 487–496.

Selected publications of the consortium :

Accadbled F, Laffosse JM, Ambard D, Gomez-Brouchet A, Sales de Gauzy J, Swider P. 2008. Influence of location, fluid flow direction, and tissue maturity on the macroscopic permeability of vertebral end plates. Spine, 33: 612–619.

Ambard D, Cherblanc F. 2009. Mechanical behavior of annulus fibrosus: a micro structural model of fibers reorientation. Annals of Biomedical Engineering, 37: 2256–2265.

Swider P, Pédrono A, Ambard D, Accadbled F, Sales de Gauzy J. 2010. Substructuring and poroelastic modelling of the intervertebral disc. J. Biomech., 43: 1287–1291.

Laffosse JM, Accadbled F, Molinier F, Bonnevialle N, Sales de Gauzy J, Swider P. 2010. Correlations between effective permeability and marrow contact channels surface of vertebral endplates. J. Orth. Res., 28: 1229–1234.

Abelin-Genevois K, Sales de Gauzy J, Briot J, Accadbled F, Swider P. 2012. Postoperative balance influences disc properties in the free motion segment in adolescent idiopathic scoliosis.47th SRS Annual Meeting USA.

Abelin-Genevois K, Estivalezes E, Briot J, Sévely A, Sales de Gauzy J, Swider P. Spino-pelvic alignment influences disc hydration properties after AIS surgery: a prospective MRI-based study. Eur Spine J. 2015; 24(6):1183-90.

CHAPTER 2

Hormones and Cytokines

The role of circulating factors, hormones or cytokines has been involved very early in the etiology of idiopathic scoliosis. In 1993 in Japan, Masafumi Machida[1], a pioneer in this field, described the relation between the absence of Melatonin (a hormone secreted in the epiphyses) and the apparition of a scoliosis in biped animals. Since then, many studies have been realized.
The current work continues to focus on melatonin and its signaling pathways, but also concentrates on other hormones such as leptin (hormone secreted by the adipic tissue) and oestrogens which could be at the origin of the predominance of scoliosis in the feminine population.
The disturbances to certain cytokines, in particular osteopontin, open promising perspectives.

[1] Machida M, Dubousset J, Imamura Y, Iwaya T, Yamada T, Kimura J. An experimental study in chickens for the pathogenesis of idiopathic scoliosis. Spine (Phila. Pa. 1976). 1993;18(12):1609-15. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8235839. Accessed December 6, 2015.

Analysis of melatonin transduction pathways and molecular expression profiles in idiopathic scoliosiss

Dr. Alain Moreau, (PhD, Montreal, Canada)) is an internationally recognized expert on molecular genetics of pediatric scoliosis. His pioneering work has led to the development of a state-of-the-art research program centered on the etiology of pediatric scoliosis. Dr. Moreau's seminal discovery stemmed from the observation of a differential signaling dysfunction of particular Gi-coupled receptors in AIS patients. This led to the stratification of AIS patients into three biological endophenotypes. Heritability was clearly demonstrated with the detection of the same endophenotype in all family members affected by scoliosis. He will coordinate the basic and clinical aspects of this project.

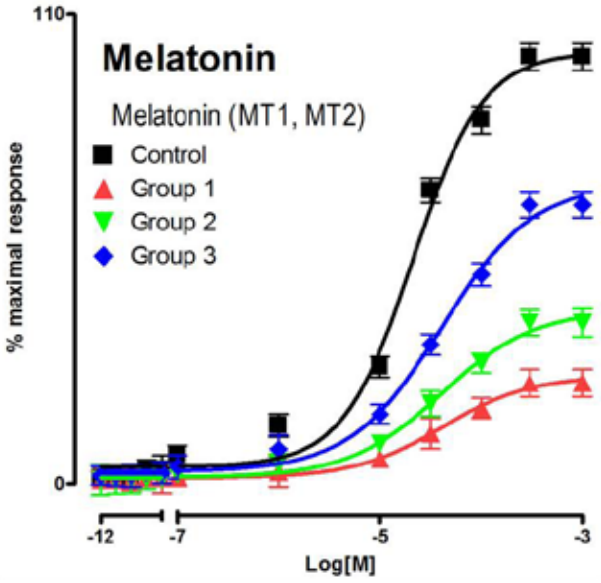
Dr. Hubert Labelle (MD, Montreal, Canada) is a clinical-scientist and orthopaedic spine surgeon who has collaborated with Dr. Moreau since 2001. He will assist Dr. Moreau in all clinical aspects.

This research project demonstrated in patients with adolescent idiopathic scoliosis (AIS) a default in the signal transmission of melatonin, a neuro-hormone that is involved in several biological processes including the regulation of the circadian cycle. This defect occurs at three proteins known as G inhibitory proteins (Gi1, Gi2 and Gi3) which are coupled to the membrane melatonin receptors (MT1 and MT2). In AIS patients, these three proteins are inactivated completely or partially following a chemical modification, called phosphorylation, occurring in different cell types including bone cells even at the level of white blood cells. This allowed us to create a classification of AIS patients in 3 groups to predict the course of their disease. More recently, we have shown that all Gi protein-coupled receptors are affected in the AIS and not just melatonin receptors MT1 and MT2. Furthermore, these discoveries led to the development of a first blood test to predict the risk of developing scoliosis in asymptomatic children as well as to predict the risk of worsening of the disease in AIS patients. Several patents have been obtained to date arising from this technology and we are looking for an industrial partner to commercialize the test.

Background :

The etiology of adolescent idiopathic scoliosis (AIS), the most common form of scoliosis, is unclear. Several divergent hypotheses have been postulated to better define this etiology (reviewed in several articles[1–5]). Genetics, growth hormone secretion, connective tissue structure, muscle structure, vestibular dysfunction, melatonin deficiency, and platelet abnormalities are major areas of research.[4] Adolescent idiopathic scoliosis affects mainly girls in number and severity, but despite the fact that several studies have suggested a genetic predisposition, the form of inheritance remains uncertain.[6–9]

The neuroendocrine hypothesis involving a melatonin deficiency as the source for AIS has generated great interest. This hypothesis stems from the fact that experimental pinealectomy in chickens, and more recently in rats, maintained in a bipedal mode, produces a scoliosis[10–13] that resembles in many aspects the human disease. Post-pinealectomy treatments with melatonin, the major hormone of the pineal gland, prevents the formation of scoliosis in both animal models.[4,10] However, the biologic relevance of melatonin in AIS is controversial because: 1) no significant decrease in circulating melatonin level has been observed in a majority of studies[14–16]; 2) experimental pinealectomy did not lead systematically to a scoliosis in all pinealectomized chickens[17–20]; and 3) melatonin



injections in pinealectomized animals did not always prevent the formation of a scoliosis.[21] Taken together, these reports raised doubts regarding the role of melatonin in AIS etiopathogenesis. These considerations led us to look instead at the melatonin signal transduction pathway because a defect of melatonin signaling activity could generate effects similar to a melatonin deficiency.

We hypothesize that idiopathic scoliosis is caused by an aberrant melatonin signaling in musculoskeletal tissues rather than by a melatonin deficiency.

Study Design

The melatonin signal transduction pathway functionality was investigated in osteoblasts from a series of patients clinically well-defined with AIS (n=41) and compared with a series of age- and gender-matched patients presenting another type of scoliosis (n= 15), including congenital scoliosis (n=3) or none (n=2).An informed consent was obtained for each patient as approved by our Institutional Ethical Committee.

Isolation of Human Osteoblasts. Osteoblasts were obtained intraoperatively from bone specimens originating from vertebrae in all cases (varying from T3 to L4 according to the surgical procedure performed) with the exception of case 41, where an iliac crest biopsy was performed on a patient with Duchenne muscular dystrophy (DMD) without a scoliosis (control patient). Bony fragments were mechanically reduced in smaller pieces with a bone cutter in sterile conditions and incubated at 37°C in 5% CO_2 in a 10-cm culture dish, in presence of á-MEM medium containing 10% fetal bovine serum (FBS; certified FBS, Invitrogen, Burlington, ON, Canada) and1% penicillin/streptomycin (Invitrogen). After a 30-day period, osteoblasts emerging from the bone pieces were separated at confluence from the remaining bone fragments by trypsinization.

Adenylyl Cyclase Activity Assay in Osteoblasts

The functionality of melatonin signaling was assessed by investigating the ability of Gi proteins to inhibit stimulated adenylyl cyclase activity in osteoblast cultures. Osteoblasts from patients with AIS and control patients were seeded in quadruplet on a 24-well plate (5 x 10^4 cells/well) and incubated with the vehicle alone, dimethyl sulfoxide (DMSO, Sigma, Oakville, ON, Canada), or forskolin (10^{-5} M, Sigma) to stimulate the cAMP formation. Inhibition curves of cAMP production were generated by adding melatonin to the forskolin-containing samples in concentrations ranging from 10^{-11} M to 10^{-5} M in a final volume of 1 mL of -MEM media containing 0.2% bovine serum albumin (BSA; Sigma).

After 30-minute incubation at 37° C, the cells were lysed and the sample centrifuged at 4° C.The cAMP content was determined in 200 ìL aliquot of the supernatant using an enzyme immunoassay kit (Amersham-Pharmacia Biosciences, Mississauga, ON, Canada). All assays were performed in duplicate. The functionality of Gi proteins was assessed by investigating their ability to inhibit adenylyl cyclase activity in osteoblasts. To obtain inhibition curves of cAMP production, the nonhydrolysable analog of GTP, Gpp(NH)p (guanilyl 5'-imidophosphate, Sigma), was added to the forskolin-containing samples in concentrations ranging from 10^{-9}M to 10^{-4}M. The cAMP content was determined as described above in similar assays with melatonin.

Results

Using multiple cell types from patients and controls, we identified a common disease mechanism among all AIS patients based on their biochemistry[22]. We developed a whole-cell assay using cellular dielectric spectroscopy to measure the activity of G-protein-coupled receptors among AIS patients and controls, and identified a G inhibitory (Gi) signaling dysfunction in AIS patients[23]. Furthermore, observations of biochemical activity led us to classify patients into three distinct groups (referred to as FG1, FG2 and FG3), depending on the maximal Gi response observed after stimulation. These biological endophenotypes can partition biological variation and thus increase the power to detect genetic associations[22, 24-26]. By definition, an endophenotype is a term from genetic epidemiology used to parse behavioral symptoms into more stable phenotypes with a clear genetic connection. Endophenotypes are believed to have a polygenic background with multifactorial origins. In complex genetic disorders such as AIS, endophenotypes have potential utility both in identifying risk genes and in illuminating the pathophysiology. The use of these endophenotypes has allowed for in-house patient stratification that has been correlated to explicit molecular expression profiles and differential prognoses, to highlight some specific clinical features of the disease. Importantly, the occurrence of severe scoliosis cases (Cobb angle e»45°) is strikingly different among each biological endophenotype. In our French–Canadian pediatric cohort, the percentages of severe cases are 13% for FG1, 60% for FG2 and 27% for FG3 endophenotypes. As in the French-Canadian cohort, the three endophenotypes occur in the Italian AIS cohort in also similar proportions of distribution in the mild-moderate stage, while FG2 was the most prevalent group in the severe stage with a proportion of 61 % compared to 36 % and 3 % for FG3 and FG1, respectively. Overall, these data indicate that the functional classification of patients may be a useful tool to predict the risk of progression of AIS but not the expression of curve type, and suggest that the need for a spinal surgery is very high for patients classified as FG2 and much less for those classified as FG1. This biochemical understanding of AIS is an important advantage for the interpretation of genetic results and has helped us to prioritize candidate genes in the context of our research program on AIS etiology.

Sources and references

1. Byrd JA, III. Current theories on the etiology of idiopathic scoliosis. Clin Orthop 1988:114-9.

2. Killian JT, Mayberry S, Wilkinson L. Current concepts in adolescent idiopathic scoliosis. Pediatr Ann 1999;28:755–61.

3. Lowe TG, Edgar M, Margulies JY, et al. Etiology of idiopathic scoliosis: current trends in research. J Bone Joint Surg Am 2000;82-A:1157–68.

4. Machida M. Cause of idiopathic scoliosis. Spine 1999;24:2576–83.

5. Veldhuizen AG, Wever DJ, Webb PJ. The aetiology of idiopathic scoliosis: biomechanical and neuromuscular factors. Eur Spine J 2000;9:178–84.

6. Axenovich TI, Zaidman AM, Zorkoltseva IV, et al. Segregation analysis of idiopathic scoliosis: demonstration of a major gene effect. Am J Med Genet 1999;86:389–94.

7. Blank RD, Raggio CL, Giampietro PF, et al. A genomic approach to scoliosis pathogenesis. Lupus 1999;8:356–60.

8. Giampietro PF, Raggio CL, Blank RD. Synteny-defined candidate genes for congenital and idiopathic scoliosis. Am J Med Genet 1999;83:164–77.

9. Wise CA, Barnes R, Gillum J, et al. Localization of susceptibility to familial idiopathic scoliosis. Spine 2000;25:2372–80.

10. Machida M, Murai I, Miyashita Y, et al. Pathogenesis of idiopathic scoliosis. Experimental study in rats. Spine 1999;24:1985–9.

11. McCarrey JR, Abbott UK, Benson DR, et al. Genetics of scoliosis in chickens. J Hered 1981;72:6–10.

12. Muccielli ML, Martinez S, Pattyn A, et al. Otlx2, an Otx-related homeobox gene expressed in the pituitary gland and in a restricted pattern in the forebrain. Mol Cell Neurosci 1996;8:258–71.

13. Wang X, Moreau M, Raso VJ, et al. Changes in serum melatonin levels in response to pinealectomy in the chicken and its correlation with development of scoliosis. Spine 1998;23:2377–81.

14. Bagnall KM, Raso VJ, Hill DL, et al. Melatonin levels in idiopathic scoliosis. Diurnal and nocturnal serum melatonin levels in girls with adolescent idiopathic scoliosis. Spine 1996;21:1974–8.

15. Brodner W, Krepler P, Nicolakis M, et al. Melatonin and adolescent idiopathic scoliosis. J Bone Joint Surg Br 2000;82:399–403.

16. Sadat-Ali M, al Habdan I, al Othman A. Adolescent idiopathic scoliosis. Is low melatonin a cause? Joint Bone Spine 2000;67:62–4.

17. Bagnall KM, Beuerlein M, Johnson P, et al. Pineal transplantation after pinealectomy in young chickens has no effect on the development of scoliosis. Spine 2001;26:1022–7.

18. Beuerlein M, Wang X, Moreau M, et al. Development of scoliosis following pinealectomy in young chickens is not the result of an artifact of the surgical procedure. Microsc Res Tech 2001;53:81–6.

19. Beuerlein M, Wilson J, Moreau M, et al. The critical stage of pinealectomy surgery after which scoliosis is produced in young chickens. Spine 2001;26: 237–40.

20. Wang X, Jiang H, Raso J, et al. Characterization of the scoliosis that develops after pinealectomy in the chicken and comparison with adolescent idiopathic scoliosis in humans. Spine 1997;22:2626–35.

21. Bagnall K, Raso VJ, Moreau M, et al. The effects of melatonin therapy on the development of scoliosis after pinealectomy in the chicken. J Bone Joint Surg Am 1999;81:191–9.

22. Akoume MY, Azeddine B, Turgeon I, Franco A, Labelle H, Poitras B, Rivard CH, Grimard G, Ouellet J, Parent S, Moreau A. Cell-based screening test for idiopathic scoliosis using cellular dielectric spectroscopy. Spine (Phila Pa 1976.). 2010;35:E601-E608.

23. Akoume MY, Franco A, Moreau A. Cell-based assay protocol for the prognostic prediction of idiopathic scoliosis using cellular dielectric spectroscopy. Journal of visualized experiments : JoVE. 2013:e50768.

24. Moreau A, Wang DS, Forget S, Azeddine B, Angeloni D, Fraschini F, Poitras B, Labelle H, Rivard CH, Grimard G. Melatonin signaling dysfunction in adolescent idiopathic scoliosis. Spine. 2004;29:1772-1781.

25. Azeddine B, Letellier K, Wang dS, Moldovan F, Moreau A. Molecular determinants of melatonin signaling dysfunction in adolescent idiopathic scoliosis. Clin.Orthop.Relat Res. 2007;462:45-52.

26. Moreau A, Akoume Ndong MY, Azeddine B, Franco A, Rompre PH, Roy-Gagnon MH, Turgeon I, Wang D, Bagnall KM, Poitras B, Labelle H, Rivard CH, Grimard G, Ouellet J, Parent S, Moldovan F. [molecular and genetic aspects of idiopathic scoliosis. Blood test for idiopathic scoliosis]. Orthopade. 2009;38:114-121.

Publications

1. **Moreau A**, Wang DS, Forget S, Azeddine B, Angeloni D, Fraschini F, Labelle H, Poitras B, Rivard C.-H, Grimard G. Melatonin signaling dysfunction in adolescent idiopathic scoliosis. Spine 2004, 29(16): 1772-1781.

2. Azeddine B, Letellier K, Wang DS, Moldovan F, **Moreau A.** Molecular determinants of melatonin signaling dysfunction in AIS. Clinical Orthopaedic & Related Research, 2007, 462: 45-52

3. Letellier K, Azeddine B, Blain S, Turgeon I, Wang DS, Samba Boiro M, Moldovan F, Labelle H, Poitras B, Rivard C-H, Grimard G, Parent S, Ouellet J, Lacroix G, and **Moreau A.** Récents progrès dans l'étiopathogenèse de la scoliose idiopathique de l'adolescent et nouveaux concepts moléculaires. Médecine/Sciences, 2007, 23(11): 910-916

Role of estrogens in adolescent idiopathic scoliosis pathogenesis

This project was initiated and performed at Sainte-Justine Hospital, in Dr Moldovan's laboratory. Dr Florina Moldovan is a full professor at Faculty of dentistry – Université de Montreal, Researcher at the Sainte-Justine University Hospital Center, which is the largest mother-child center in Canada and one of the four most important paediatric centers of all North America.

The research team of this project was composed of basic scientists, clinicians and students. Group of three Orthopaedic Surgeons (Dr Guy Grimard, Dr Stefan Parent and Dr Hubert Labelle), Dr Moreau Alain (PhD), two young scientists (Dr Kessen Patten and Dr Khaled Fendri both post-doctoral students), and two MSc students, (Dominique Leboeuf and Kareen Letellier), has played a key role in developing this research project.

Our research work connected particular genes and pathways important in AIS: the developmental/growth-differentiation of skeletal elements, cellular signaling connecting structural integrity of the extracellular matrix to the structural integrity of bone, cartilage and muscles. We identified differentially expressed genes responsible for AIS (differentially expressed with embryogenesis/morphogenesis), while other genes are more related to spinal deformity progression. In conclusion, we identified the mechanism by which estrogen get involved in AIS. Consequently, our results add a new facet to the understanding of the role and function of hormons in AIS and revealed new potential contribution pathways for AIS. Taken together, this study has demonstrated gene expression changes in osteoblasts from AIS patients, as compared to osteoblasts from healthy controls. The reported results help to gain further insight into potential genes and molecular pathways that could contribute to understanding the pathophysiology of idiopathic scoliosis. Identification of the underlying mechanisms that lead to the observed clinical features of scoliosis remains the crucial step to further advance understanding of AIS pathogenesis.

Background

Several physio-pathological and clinical observations suggest that estrogens could play a critical role in the pathogenesis of AIS, but the precise mechanisms involved are unclear. In fact, the increased occurrence of scoliosis in girls is prominent; clinically significant spinal deformities (of at least 10°of deformity) are found twice as often in girls, and this ratio increases to 8:1 for scoliosis of more than 30° in the frontal plan (Weinstein et al 2008). In patients with scoliosis, puberty appears to be a period of «dangerous» growth. This is highlighted by a curve describing scoliosis progression as initially explained by Duval-Beaupère (Duval-Beaupere 1982,1996). This curve illustrates that the progression of the spinal deformity in AIS is strongly dependent on growth and bone maturation, (highly influenced by estrogens). Their exact role has not yet been explained, but it is well known that scoliosis curve progression occurs during skeletal growth (Sarvark et al 2007) and that estrogens are uniquely responsible for the growth and spinal maturity [Sanders et al 2007]. Thus, clarifying the role of estrogens is essential for understanding how AIS evolves during the skeletal growth.

Hypothesis

The goal of this project was to clarify the role of estrogens and estrogens receptors in the Adolescent Idiopathic Scoliosis (AIS). We tried to understand why AIS affects girls and why this disease progresses during the puberty growth spurt. Indeed, we advanced the hypothesis that estrogens interplay with other factors, such as the failure of the melatonin pathways. To look at a more global picture of cellular function in osteobalsts, we also conducted a microarray gene profiling analysis.

This study examined genes with different expressions in AIS compared to the normal patients. Several tissues from normal (trauma) and AIS patients were collected and Tissues Bank was created in collaboration with Dr. Alain Moreau's team.

Results

Thanks to the Yves Cotrel Foundation, the research of Dr Moldovan's laboratory has shown that estrogens play a critical role in AIS (Letellier et al 2008, Leboeuf et al 2009) through their impact on bone cell signaling and function. These published results indicate that estrogens are not at the origin of AIS; however, they interplay with the osteoblast signaling defect in AIS patients, and this in turn could interact with defective genes or gene products that remain to be identified and functionally validated. We yield a list of genes, and related potential pathways, differentially expressed in AIS cells, and associated with embryogenesis/ morphogenesis) (Fendri et al 2013).

We also clarified the role of chromodomain helicase DNA-binding protein 7(CHD7 gene) in AIS. For instance, a polymorphism in the CHD7 has been associated with susceptibility to idiopathic scoliosis in humans (Gao et al 2007). Indeed, in Patten et al 2012 we established a Chd7 knockdown in zebrafish, and reported essential role of Chd7 in retinal organization and the development of photoreceptors, axial patterning, and bone development and mineralization. This was the first study to report the requirement of Chd7 in retinal and vertebral development, but without any skeletal defects reminiscent of scoliosis. In Letellier et al 2008, we reported the crosstalk between estrogens and melatonin in AIS. We demonstrated an abnormal balance in the Gái and GSá protein ratio promoted by estrogen–melatonin crosstalk in AIS cells. The importance of this study lies in the fact that estrogens might correct the melatonin-signaling defect by decreasing cAMP production in a specific group of AIS patients, but not in all patients. From a clinical point of view, this indicates that late menarche (which is observed frequently in scoliotic patients) could take part in spinal deformity aggravation. In Leboeuf et al 2009, we have examined the possible role of estrogens in AIS progression. For instance, we suggested a new avenue of pharmacological interventions to stop AIS aggravation during skeletal growth, once the genetic determinants of AIS and optimal skeletal development at puberty will be elucidated. Using microarray expression profiling, our recent study (Fendri et al 2013) identifies genes with an altered expression in AIS (Fig. 1 Eur Spine J 2013; 22(6): 1300-1311). This publication has been selected as the best basic science paper in the European Spine Journal in the past year («GRAMMER European Spine Journal Award 2014»). In this project, we screened candidate genes that may contribute to the AIS. Indeed, our study provides a previously unrecognized list of genes belonging to the same family or clearly connected, that merit further investigation, as AIS contributive genes.

In conclusion, our results add a new facet to the understanding of the role and function of estrogens and melatonin in AIS, and revealed new potential contribution pathways for AIS.

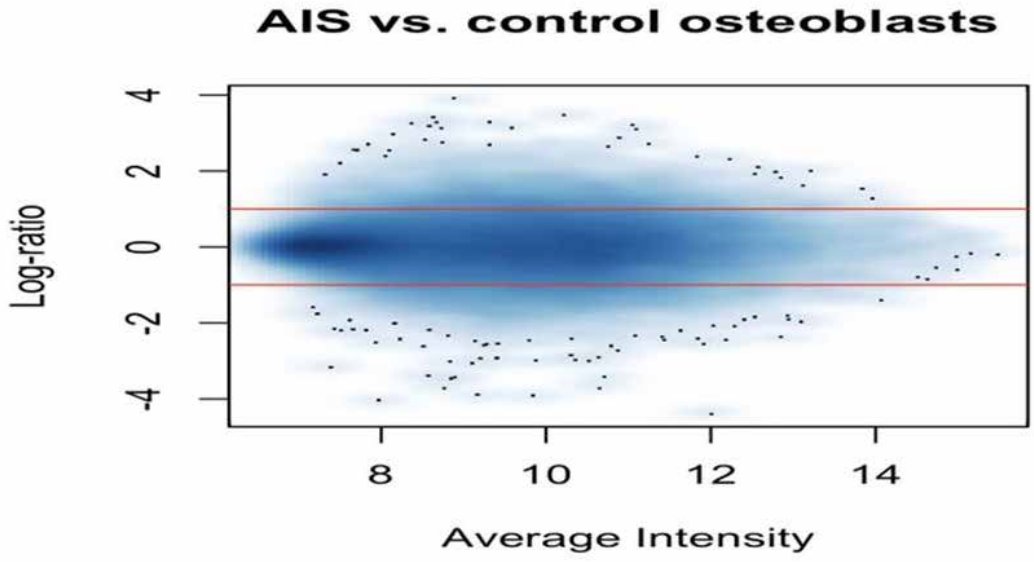


Figure 1: *Gene Expression Profiling by Microarray-based Analysis identifies genes whose expression was altered in adolescent Idiopathic scoliosis compared to the normal (control) patients. (Fendri et al, Eur Spine J 2013)*

Sources and references

Weinstein, S.L. et al. (2008) Adolescent idiopathic scoliosis. Lancet 371, 1527–1537

Sarwark, J. and Aubin, C.E. (2007) Growth considerations of the immature spine. J. Bone Joint Surg. Am. 89 (Suppl 1), 8–13

Sanders, J.O. et al. (2007) Maturity assessment and curve progression in girls with idiopathic scoliosis. J. Bone Joint Surg. Am. 89, 64–73

Duval-Beaupere G. Surveillance, course, and prognosis of scoliosis. Rev Prat. 1982 Nov 11;32(51-52):3261-4, 3269-71

Duval-Beaupere G. Threshold values for supine and standing Cobb angles and rib hump measurements: prognostic factors for scoliosis. Eur Spine J. 1996;5(2):79-84.

List of publications supported by the FondationYves Cotrel Foundation - Institut de France

Letellier K, Azeddine B, Blain S, Turgeon I, Wang da S, Boiro MS, **Moldovan F,** Labelle H, Poitras B, Rivard CH, Grimard G, Parent S, Ouellet J, Lacroix G, Moreau A Etiopathogenesis of adolescent idiopathic scoliosis and new molecular concepts]. Med Sci (Paris). 2007 Nov;23(11):910-6. Review. French.

Azeddine B, Letellier K, Wang da S, **Moldovan F,** Moreau A. Molecular determinants of melatonin signaling dysfunction in adolescent idiopathic scoliosis. Clin Orthop Relat Res. 2007 Sep;462:45-52.

Letellier K, Azeddine B, Parent S, Labelle H, Rompré PH, Moreau A, **Moldovan F**.J Pineal Res.Estrogen cross-talk with the melatonin signaling pathway in human osteoblasts derived from adolescent idiopathic scoliosis patients. 2008 Nov;45(4):383-93. doi: 10.1111/j.1600-079X.2008.00603.x.

Leboeuf D, Letellier K, Alos N, Edery P, **Moldovan F.** (2009) Do estrogens impact adolescent idiopathic scoliosis? Trends in Endocrinology and Metabolism (IF 7.1) Trends Endocrinol Metab. 2009 May;20(4):147-52.

Edery P, Margaritte-Jeannin P, Biot B, Labalme A, Bernard JC, Chastang J, Kassai B, Plays MH, **Moldovan F**, Clerget-Darpoux F. New disease gene location and high genetic heterogeneity in Idiopathic Scoliosis. Eur J Hum Genet. 2011 Aug; 19(8):865-9.

Fendri K, **Moldovan F.** Potential role of COMP as a biomarker for idiopathic scoliosis. Med Hypotheses, 2011 May; 76(5):762-3.

Patten S, **Moldovan F.** Could genetic determinants of inner ear anomalies be a factor for the development of idiopathic scoliosisis? Med Hypotheses. 2011 Mar; 76(3):438-40.

Patten S, Jacobs-McDaniels NL, Zaouter C, Drapeau P, Albertson RC, **Moldovan F**. Role of Chd7 in zebrafish: a model for CHARGE syndrome. PLoS One. 2012; 7(2):e31650.

Acaroglu E, Bobe R, Enouf J, Marcucio R, **Moldovan F**, Moreau A. The metabolic basis of adolescent idiopathic scoliosis: 2011 report of the 'metabolic' workgroup of the Fondation Yves Cotrel. Eur. Spine J. 2012 Jun; 21(6):1033-4.

Shunmoogum A. Patten, Patricia Margaritte-Jeannin, Jean-Claude Bernard, Eudeline Alix, Audrey Labalme, Alicia Besson, Simon L. Girard, Khaled Fendri, Nicolas Fraisse, Bernard Biot, Coline Poizat, Amandine Campan-Fournier, Kariman Abelin-Genevois, Vincent Cunin, Charlotte Zaouter, Meijiang Liao, Raphaelle Lamy, Gaetan Lesca, Rita Menassa, Charles Marcaillou, Melanie Letexier, Damien Sanlaville, Jerome Berard, Guy A Rouleau, Françoise Clerget-Darpoux, Pierre Drapeau, **Florina Moldovan***, Patrick Edery*. Functional variants of the POC5 gene in Idiopathic Scoliosis. J Clin Invest. 2015 Feb 2. pii: 77262. doi: 10.1172/JCI77262

The metabolic basis of Adolescent Idiopathic Scoliosis

Emre R. Acaroglu was born in Ankara Turkey.
He earned his MD degree in 1986 and started residency in the Dpt of Orthopedics and Traumatology in Hacettepe University. He was appointed as an assistant professor in December 1992.
He did his spine fellowship in New York from 1993 to 1994 (Columbia University, directed by Drs. JP Farcy and M Weidenbaum) with a parallel research appointment in the Orthopedic Research Laboratory, directed by Mr. V. Mow PhD.
He earned the title of Associate Professor in 1995, received tenure in 1996 and became a Full Professor of Orthopedics and Traumatology in 2002. He spent 15 months at the University of California San Francisco from 2003 and 2004 as a sabbatical. He served as the chief of spinal surgery in Hacettepe University Department of Orthopedics and Traumatology till 2009.
Currently he is the director of Ankara Spine Center and chief of Orthopedic Spine. Dr. Acaroglu clinically specializes in Adult and Pediatric Spinal Deformity. He maintains a keen interested in medical education.
His research interest and activities encompass a range of clinical as well as basic research projects. He is an executive member of an international study group that focuses on adult deformity (European Spine Study Group) and has obtained research funding from the European Spine Society in connection with this study group. His research on the pathogenesis of adolescent idiopathic scoliosis in collaboration with scientists from UCSF and U of Montreal is funded by the SRS and the Cotrel Foundation.
He is the co-founder and Director of Acibadem ARTES Spine Center in Ankara.

Ralph Marcucio began his research career as an intern at The Boyce Thompson Institute while he was an undergraduate at Cornell University in Ithaca, NY.
He completed his PhD at Cornell University in 1995, and was awarded a prestigious NIH training grant to study development of the musculoskeletal system. In 2000, Dr. Marcucio joined the Molecular and Cellular Biology Laboratory at the University of California, San Francisco, (UCSF), and in 2003, he was appointed to the faculty at UCSF in the Department of Orthopaedic Surgery. Dr. Marcucio's research focuses on various aspects of bone biology including mechanisms of scoliosis, bone regeneration, and morphogenesis of the facial skeleton.

Our studies consisted of three different projects (four experiments) to help define the significance of melatonin and calmodulin in the pathogenesis and mechanisms

Project 1 : Comparison of the Expressions of Melatonin, Calmodulin and 5-HT4 in Paravertebral Muscle and Platelets of Patients With or Without Adolescent Idiopathic Scoliosis

In the first part, tissue levels of melatonin and calmodulin were measured in platelets and skeletal muscle derived from patients with adolescent idiopathic scoliosis undergoing surgical treatment and compared to a group of patients with no underlying chronic spinal problem . Results of this study revealed that contrary to previously published reports, tissue calmodulin levels were not significantly different in scoliotics compared to controls, a finding that indicates platelet calmodulin levels may not be adequate to use as a screening tool for the impending onset of scoliosis.

Likewise, tissue melatonin concentrations were not significantly different in scoliotics compared to controls. Based on our findings, it may be postulated that in accordance with the studies demonstrating no differences in serum levels, there are probably no differences in tissue levels as well. Furthermore, it was also demonstrated that skeletal muscle calmodulin content was significantly different when the convex side was compared to the concave side. However, contrary to previous findings, our results showed an increase of muscle calmodulin levels at the convex side. This is an important finding as it demonstrates that calmodulin may be involved in the regulation of contraction of skeletal muscles and electrophysiological differences between these muscles may reflect differences in calmodulin concentration.

Although calmodulin is probably not a causative factor, this molecule may be one of the important factors contributing to the progression of scoliotic curves. In other words, it may be hypothesized that calmodulin is probably not the factor triggering the occurrence of deformity but the secondary imbalance in calmodulin content may be the factor governing progression.

Project 2 : The effect of calmodulin inhibitors on scoliosis in C57Bl6 mice : Refining the timing of intervention, dosage, gender differences and histopathological mechanisms

a) We have tested the involvement of calmodulin in the pathogenesis of IS in two other studies on animal models, a pinealectomized chicken model and a C57Bl6 mice model, attempting to antagonize calmodulin using two different CaM antagonist pharmacological agents, tamoxifen (TMX) and trifluoperazine (TFP) (3, 4). Findings of these studies demonstrated that as hypothesized, Calmodulin inhibitors do not prevent the occurrence of scoliotic deformities in either the pinealectomized chicken or the C57BL6 mice models. On the other hand, tamoxifen was shown to decrease the rate of progression of deformity in both models. This observation suggests that our hypothesis on the influence of calmodulin on the progression of the deformity may be correct. In addition, in both models, tamoxifen with or without the addition of trifluoperazine led to the reversal of curvature in a significantly higher number of animals compared to controls. Interestingly, tamoxifen and raloxifen appear to have a negative effect on the smooth muscle tone of vascular walls as well and their mechanism of action in the reversal of deformity in animal models may be based on this effect. However, it has to be stressed that the action of tamoxifen might not have necessarily been based on its affect on calmodulin but by way of a different interaction, specifically the regulatory effect on estrogen or estrogen regulated proteins.

b) The clinical association of AIS with osteopenia was brought to attention by the works of Cheng and coworkers. Although it was assumed that this finding may be the consequence of a problem in the vitamin D synthesis or metabolism, no defects in this system or appropriate genetic polymorphisms could be identified .

Our unpublished pilot data on the bipedal C57Bl6 model also suggests that the animals with scoliosis have significantly less trabecular density compared to those animals that received tamoxifen treatment. In another study we demonstrated that raloxifen may be as effective in the reversal of scoliotic deformity in C57Bl6 mice as tamoxifen.

Based on these observations, we did an experiment to identify whether osteopenia might be a primary factor in the development of scoliosis in a bipedal osteoporotic rat model .

Our findings demonstrate that in spite of the fact that these animals were not pinealectomized, 65% of the control animals and 82% of the osteopenic animals developed measurable scoliotic curves with average magnitudes of 10,0 ± 4,3 and 11,8 3,75 respectively ($p>0,05$), implicating that contrary to our hypothesis

osteopenia is probably not a primary factor.
However, these findings do suggest that selective estrogen receptor modulators (SERM) such as tamoxifen and raloxifen may be effective in the reversal of osteopenia and scoliotic deformity in animal models. In other words, although the experiments with these molecules were started with the assumption that their CaM antagonism had been the mechanism of action, these findings suggest that estrogen receptor modulation may be the key factor in the observed effects, especially considering that raloxifen probably does not have any anti-CaM properties.

The above observations suggest that estrogen and/or estrogen receptors may be key factors in the pathogenesis of AIS. This assumption indeed makes clinical sense as it may explain the sexual predilection associated with the disease. Furthermore, it may also explain the observation of osteopenia in scoliotic individuals, potentially similar to the osteopenia observed following menopause. Estrogen receptor gene polymorphism has been recognized to be associated

Project 2 : The pathogenesis of «idiopathic scoliosis» modeled as a combination of growth, osteopenia and bipedality. A study on bipedal C57Bl6 mice model (*not finalized*)

In collaboration with Gokhan Demirkiran MD and Florina Moldovan MD PhD

This is a project attempting to find a medical treatment for progressive scoliosis

with AIS in humans by works of Inoue and by Moldovan and coworkers .

The specific aims of this project were

·To identify the tridimensional effects of bipedality on spinal column including pelvis and to correlate these findings with the incidence and severity of scoliosis as well as histomorphological and some biochemical parameters pathologies.

· To analyze the timing of occurrence of these histomorphological and biochemical parameter pathologies in vertebral bone, and growth plates, brain, and muscles and to correlate these with the timing of occurrence of scoliotic deformity.

· To analyze the growth plate and vertebral body changes in relation to left/right and front/back asymmetries and to correlate these with the tridimensional structural changes imposed by bipedality.

· To analyze the effects of several pharmacological agents effective on the proposed mechanism such as TMX, RLX, phytoestrogens (lignan) estrogen and NO donors on the histomorphometric and 3D structural parameters as well as the occurrence and severity of experimental scoliosis. · To validate the findings in humans in an animal model that may be used for testing treatment using various pharmacological agents as stated above.

Results

Radiological analysis :

On coronal deformity analysis, scoliosis incidences by groups and time points revealed that the overall rise of the incidence to the 20th week was similar in all groups (including quadripeds) but was followed by a decrease in Rlx and E2 groups after 20 weeks. There was a regular increase in scoliosis incidence in NO group. The median of the curve degrees at 20th week and 40th week were highest at estrogen (p=0.066) group and quadripedal group (p=0.562) respectively.

As for sagittal analysis; Results demonstrated that the quadripedal group has lower kyphosis and higher lordosis and SSA at all times (significant only at 20th week). Bipedal group started with a lower pelvic incidence but developed increasingly and significantly

Histology : Trichrome staining and Immunehistochemistry (IHC)

Trichrome analysis suggests that the NPs of IVDs in bipedal animals may be smaller in proportion compared to quadripedals. Likewise, the bipedal IVDs demonstrated significantly higher levels of ER beta and calmodulin receptors but the difference in ER alpha receptors was not significant. None of the studies have demonstrated any significant changes between the study groups.

Several deductions may be made based on these results

Scoliosis incidence in this study was significantly higher than our previous studies. Of note, even the quadripedal animals developed scoliosis, with the highest rate as well. This is contrary to the results of previous studies by others but suggest that bipedality may not be an essential factor in the development of scoliosis in this model.

As previously demonstrated by us, administration of RLX is moderately effective in preventing scoliosis progression. Likewise, new to this study, E2 administration has proved to be mildly effective as well. NGly could not be shown to have any effect in this respect.

Bipedality is associated with a larger pelvis (increased PI) and some decrease in TK and SSA. These effects so far have not proved to be as pronounced as we had hypothesized but nevertheless do exist. Further analysis along with the 3D CT data may prove to be more useful in understanding the real relevance of these findings.

The main histology and IHC differences appear to be between bipedal and quadripedal animals, independent of the administration of pharmacological agents. We would like to exercise caution in attributing any relevance to this finding at this stage and await the complete results of histology and histomorphometric analysis.

Publications

1. Demirkiran G, Dede O, Yalcin N, Akel I, Marcucio R, Acaroglu E. Selective

Estrogen receptor modulation prevents scoliotic curve progression: radiologic and histomorphometric study on a bipedal C57Bl6 mice model. Eur Spine J. 2014 Feb; 23(2):455-62. doi: 10.1007/s00586-013-3072-2. Epub 2013 Oct 18. PubMed PMID: 24136418; PubMed Central PMCID: PMC3906449.

2. Acaroglu E, Bobe R, Enouf J, Marcucio R, Moldovan F, Moreau A. The metabolic basis of adolescent idiopathic scoliosis: 2011 report of the «metabolic» workgroup of the Fondation Yves Cotrel. Eur Spine J. 2012 Jun;21(6):1033-42. PubMed PMID: 22402844; PubMed Central PMCID: PMC3366135.

3. Dede O, Akel I, Demirkiran G, Yalcin N, Marcucio R, Acaroglu E. Is decreased bone mineral density associated with development of scoliosis? A bipedal osteopenic rat model. Scoliosis. 2011 Oct 31;6(1):24. PubMed PMID: 22040734; PubMed Central PMCID: PMC3217908.

4. Acaroglu E, Akel I, Alanay A, Yazici M, Marcucio R. Comparison of the

melatonin and calmodulin in paravertebral muscle and platelets of patients with or without adolescent idiopathic scoliosis. Spine (Phila Pa 1976). 2009 Aug 15;34(18):E659-63. PubMed PMID: 19680092.

5. Akel I, Kocak O, Bozkurt G, Alanay A, Marcucio R, Acaroglu E. The effect of

calmodulin antagonists on experimental scoliosis: a pinealectomized chicken model. Spine (Phila Pa 1976). 2009 Mar 15;34(6):533-8. PubMed PMID: 19282733.

6. Akel I, Demirkiran G, Alanay A, Karahan S, Marcucio R, Acaroglu E. The effect of calmodulin antagonists on scoliosis: bipedal C57BL/6 mice model. Eur Spine J. 2009 Apr;18(4):499-505. PMID: 19242737; PubMed Central PMCID: PMC2899472.

Study of tyrosine phosphatase RPTPμ and osteopontin in AIS etiopathogenesis

Dr. Alain Moreau, (PhD, Montreal, Canada)

This research project demonstrated in patients with adolescent idiopathic scoliosis (AIS) that elevated blood levels of a molecule called osteopontin (OPN), which plays many biological roles including a role in inflammation. Our work in several animal models (chickens, mice and zebrafish) have demonstrated that OPN is the responsible factor in the development of scoliosis and high OPN levels in the blood of AIS patients contributes to advance their spinal deformities. It should be noted, that circulating OPN levels are variable in function of certain genetic predispositions as well as in response to environmental factors (mycobacteria, diet, medication, strength, etc.). We also discovered that several other molecules could have a positive or negative effect in the response of cells to OPN in AIS. Indeed, mice lacking the gene Ptprm encoding for the tyrosine phosphatase RPTPì are more severely affected and all developed a severe scoliosis. Some experimental evidence suggests that this effect is mediated by an increased binding of OPN to a specific receptor and that OPN signaling is causing the Gi-coupled receptor signaling impairment. Furthermore, these discoveries led to the development of a biochemical test to predict the risk of disease progression in AIS patients. Several patents have been obtained to date arising from this technology and we are looking for an industrial partner to market it.

Background

Idiopathic scoliosis is a three-dimensional deformity of the spine with lateral curvature accompanied by a vertebral rotation (1). Despite considerable advances in research involving this disease, its aetiology and pathogenesis remain unclear (2), (3), (4). From the current understanding, it is generally accepted that the cause of idiopathic scoliosis is multifactorial (2), (3), (4) and an emerging concept indicates that a biological process driving the pathogenesis may implicate defective GiPCR signaling (5), (6), (7) (8).

Recently, we have identified OPN, a multifunctional glycoprotein, as a potentially key pathophysiologic contributor in the development of idiopathic scoliosis (9), (unpublished data). Particularly, we have documented increased plasma OPN in patients with idiopathic scoliosis and in bipedal mice, a well-established animal model of this disease. Furthermore, using OPN knockout mice, we demonstrated that a lack of OPN protects bipedal mice against scoliosis and improves GiPCR signaling in their osteoblasts. We further showed that OPN reduces GiPCR signaling in *vitro* via a dual mechanism involving â1 integrin engagement.

It is known that the engagement of integrins by ligands is facilitated by the talin binding that allows integrins to switch from the low affinity to the high affinity form for ligands (10), (11), (12). Previously, talin binding to integrin was shown to be modulated by PIPK1γ .This enzyme generates the lipid second messenger phosphatidylinositol-4,5 biphosphate (PI4,5P2), which promotes the interaction of talin with integrin (13). Tyrosine phosphorylation of PIPK1γ upon FAK-dependent Src activation is essential for this interaction since it stimulates the catalytic activity of this enzyme, resulting in enhanced PI4,5P2 production in the vicinity of integrin and further promotion of talin binding to integrin (14), (15). Termination of this process occurs following dephosphorylation by various tyrosine phosphatases among which PTPμ has been identified as the most promising for maintaining PIPK1γ in a hypophosphorylated state (16).

Hypothesis

We hypothesized that scoliosis onset and spinal deformity progression are both triggered by a biological pathway involving Osteopontin (OPN), a multifunctional secreted cytokine, and its signaling action through integrins. Based on these considerations cited above, we hypothesized that the lack of PTPμ may exacerbate the inhibitory effect of OPN on GiPCR signaling and influence the nature of scoliosis. Our results show that the genetic deletion of PTPμ enhances the incidence and severity of scoliosis, possibly by favoring the interaction of OPN with β1 integrin. This study highlights the importance

of the inhibitory effect of OPN on GiPCR signaling in the pathogenesis of idiopathic scoliosis

Methodology

Animals: Breeding pairs of PTPμ knockout mice on the CB57BL/6 background were a generous gift of Dr. Gebbink MF (University Medical Center Utrecht, Utrecht, the Netherlands), and CB57BL/6 Wild type mice were purchased from Charles River Laboratories (St. Constant, Qc Canada).
The animals were bred as separate colonies in a pathogen-free facility maintained at 25 °C on a 12-h light/dark cycle. Each animal had free access to food and water.
Bipedal conditions were induced in 4-weeks old mice by amputation of the forelimbs and tails under anaesthesia as previously described (17). From 2 weeks post-surgery, mice were screened for scoliosis every two weeks for 36 weeks using a Faxitron X-rays apparatus (Faxitron X-rays Corp. Wheeling, IL, USA). Procedures were approved by the Institutional Animal Care and Use Committee of the Université de Montréal and McGill University and were in accordance with the Canadian Council on Animal Care (CCAC) policy for the care and use of laboratory animals.

Blood collection and OPN measurement: Peripheral blood from anesthezed mice or from human patients were collected into EDTA-treated tubes and plasma was then separated by centrifugation at 3 000 rpm for 10 min at 4 °C and stored at -80 °C. Plasma OPN protein concentrations were measured using a specific ELISA kit from IBL (Hamburg, Germany) according to the manufacturer's instructions. All OPN measurements were perfor-med in duplicate, using an AsysHiTech Expert-96 microplate reader from Biochrom (Cambridge, UK).

Cell culture: Osteoblasts were prepared as previously described (5), and maintained in α-modified minimum essential medium (α-MEM) supplemented with 10% foetal bovine serum (FBS) and 1% penicillin/streptomycin under standard conditions (37°C / 5% CO2). Culture media was renewed every three days and cells were allowed to grow until confluence. All compounds of the cell culture media were from Invitrogen.

siRNA transfection: Cells were transiently transfected with appropriate siRNA in serum-free medium, using Lipofectamine RNAiMAX reagent (Invitrogen) according to the manufacturer's instructions. The cells were harvested for RNA extraction after 48hrs and the gene knockdown was verified by quantitative real-time PCR (qPCR). siRNA for the knockdown of OPN or phosphatidylinositol-phosphate kinase type I gamma (PIPK1γ) as well as scrambled siRNA, were obtained from Ambion (Ambion USA).

Quantitative reverse transcription-polymerase chain reaction (qPCR): Thermo-Script reverse transcriptase (Invitrogen) was used to reverse mRNA into cDNA (1mg total concentration). Each amplification was performed in duplicate using 5ml of diluted cDNA, 7,5ml of 3mM primer solution and 12,5ml of 2X QuantiTect SYBR Green PCR Master Mix (QIAGEN Inc, Ontario, Canada). All reaction mixes were run on Mx3000P system from Stratagene (Agilent Technologies Company, La Jolla, CA) and analyzed with MxPro QPCR Software also from Strata-gene. Relative quantification was calculated with the delta CT method using α-actin as the endogenous control.

Immunoprecipitation and Western blot: Cells were lysed in RIPA buffer (25 mM Tris.Hcl pH7.4, 150 mM NaCl, 1 % NP-40, 1 % sodium deoxycholate, 0.1 % SDS) containing 5 mM $NaVO_4$ and protease inhibitor cocktail (Roche molecular Biochemicals, Mannheim, Germany). For immunoprecipitation, lysates were first pre-cleared with 25 μl of protein sepharose (A) beads (GE Healthcare Bioscience AB, Canada). The supernatant was then incubated with appropriate antibodies, followed by 1h incubation with protein G beads with gentle rocking. The beads were washed three times with lysis buffer and bound proteins were eluted with gel loading dye and boiled at 100° C for 5 minutes, before being separated on 10 % SDS-PAGE and blotted to nitrocellulose. The supernatant was then incubated with anti- PTPμ antibody (SC-25433), (Santa Cruz Biotechnology Inc., Santa Cruz, CA) or anti- OPN antibody (sc10593; Santa Cruz Biotechnology, Inc) , followed by 1h incubation with protein A beads with gentle rocking. The beads were washed three times with lysis buffer and bound proteins were eluted with gel loading dye and boiled at 100° C for 5 minutes, before being separated on 10 % SDS-PAGE and blotted to nitrocellulose. The immune complexes were analyzed by Western blot analysis with antibody against integrin β1 (SC-6622), integrin β3 (SC-6627), integrin β5 (SC-5401), integrin α4 (sc- 6589), integrin α5 (sc-166681), integrin α8 (sc-30983) (Santa Cruz Biotechnology Inc., Santa Cruz, CA) or integrin αv (4711) (Cell signaling technology, Ontario, Ca). Immunoreactivity was visualized using the SuperSignal West Pico Chemilunescent Substrate

Results obtained :

Contribution of Osteopontin (Spp1) to scoliosis onset and spinal deformity progression.

Recently, we have identified OPN, a multifunctional glycoprotein, as a potentially key pathophysiologic contributor in the development of idiopathic scoliosis. Particularly, we have documented increased plasma OPN in patients with idiopathic scoliosis and in bipedal mice, a well-established animal model of this disease. Furthermore, using OPN knockout mice, we demonstrated that a lack of OPN protects bipedal mice against scoliosis and improved GiPCR signaling in their osteoblasts. We further showed that OPN reduces GiPCR signaling in vitro via a dual mechanism involving β1 integrin engagement (manuscript to be submitted to Science Signaling).

Contribution of PTPmu in AIS pathogenesis.

Our studies suggest that osteopontin (OPN) plays a critical role in the development of idiopathic scoliosis by reducing Gi protein-coupled receptor (GiPCR) signaling via β1 integrin engagement. Here, we establish a link between the severity of scoliosis associated with OPN action and the lack of protein tyrosine phosphatase μ (PTPμ), a negative regulator of integrin activation. We demonstrate that genetic deletion of PTPμ enhances the incidence and severity of scoliosis without affecting plasma levels of OPN or the expression of its receptors. In contrast, increased interaction of OPN with β1 integrin was notified in cells depleted of PTPμ. Furthermore, a greater reduction of GiPCR signaling by OPN was also observed in these cells, while their response to GiPCR stimulation was improved with siRNA of phosphatidylinositol-phosphate kinase type I gamma (PIPK1γ), a PTPμ substrate that favours ligand biding to integrin. These studies provide the first indication that the loss of PTPμ influences the nature of idiopathic scoliosis, possibly by amplifying the inhibitory effect of OPN on GiPCR signaling (Elbakry et al. 2015 manuscript in preparation).

Sources and references

1. Kane, W. J. (1977) Scoliosis prevalence: a call for a statement of terms. Clinical orthopaedics and related research, 43-46

2. Dickson, R. A. (1992) The etiology and pathogenesis of idiopathic scoliosis. Acta orthopaedica Belgica 58 Suppl 1, 21-25

3. Machida, M. (1999) Cause of idiopathic scoliosis. Spine 24, 2576-2583

4. Burwell, R. G. (2003) Aetiology of idiopathic scoliosis: current concepts. Pediatric rehabilitation 6, 137-170

5. Moreau, A., Wang, D. S., Forget, S., Azeddine, B., Angeloni, D., Fraschini, F., Labelle, H., Poitras, B., Rivard, C. H., and Grimard, G. (2004) Melatonin signaling dysfunction in adolescent idiopathic scoliosis. Spine 29, 1772-1781

6. Azeddine, B., Letellier, K., Wang da, S., Moldovan, F., and Moreau, A. (2007) Molecular determinants of melatonin signaling dysfunction in adolescent idiopathic scoliosis. Clinical orthopaedics and related research 462, 45-52

7. Akoume, M. Y., Azeddine, B., Turgeon, I., Franco, A., Labelle, H., Poitras, B., Rivard, C. H., Grimard, G., Ouellet, J., Parent, S., and Moreau, A. (2010) Cell-based screening test for idiopathic scoliosis using cellular dielectric spectroscopy. Spine 35, E601-608

8. Akoume, M. Y., Franco, A., and Moreau, A. (2013) Cell-based assay protocol for the prognostic prediction of idiopathic scoliosis using cellular dielectric spectroscopy. Journal of visualized experiments : JoVE, e50768

9. Moreau, A., Akoume Ndong, M. Y., Azeddine, B., Franco, A., Rompre, P. H., Roy-Gagnon, M. H., Turgeon, I., Wang, D., Bagnall, K. M., Poitras, B., Labelle, H., Rivard, C. H., Grimard, G., Ouellet, J., Parent, S., and Moldovan, F. (2009) [Molecular and genetic aspects of idiopathic scoliosis. Blood test for idiopathic scoliosis]. Der Orthopade 38, 114-116, 118-121

10. Tozer, E. C., Hughes, P. E., and Loftus, J. C. (1996) Ligand binding and affinity modulation of integrins. Biochemistry and cell biology 74, 785-798

11. Hynes, R. O. (2002) Integrins: bidirectional, allosteric signaling machines. Cell 110, 673-687

12. Calderwood, D. A. (2004) Integrin activation. Journal of cell science 117, 657-666

13. Martel, V., Racaud-Sultan, C., Dupe, S., Marie, C., Paulhe, F., Galmiche, A., Block, M. R., and Albiges-Rizo, C. (2001) Conformation, localization, and integrin binding of talin depend on its interaction with phosphoinositides. The Journal of biological chemistry 276, 21217-21227

14. Di Paolo, G., Pellegrini, L., Letinic, K., Cestra, G., Zoncu, R., Voronov, S., Chang, S., Guo, J., Wenk, M. R., and De Camilli, P. (2002) Recruitment and regulation of phosphatidylinositol phosphate kinase type 1γ by the FERM domain of talin. Nature 420, 85-89

15. Ling, K., Doughman, R. L., Iyer, V. V., Firestone, A. J., Bairstow, S. F., Mosher, D. F., Schaller, M. D., and Anderson, R. A. (2003) Tyrosine phosphorylation of type Iγ phosphatidylinositol phosphate kinase by Src regulates an integrin–talin switch. The Journal of cell biology 163, 1339-1349

16. Sakamoto, Y., Ogita, H., Komura, H., and Takai, Y. (2008) Involvement of nectin in inactivation of integrin αvβ3 after the establishment of cell-cell adhesion. Journal of Biological Chemistry 283, 496-505

Publications:

1. Lombardi G, Akoume M-Y, Colombini A, Moreau A, Banfi G. Biochemistry of Adolescent Idiopathic Scoliosis. Advances in Clinical Chemistry 2011; 54: 165-82.

2. Elbakry M, Taheri M, Bouhanik S, Akoume M, Moreau A. Identification of key disease-modifying factors in adolescent idiopathic scoliosis. Stud Health Technol Inform. 2012; 176:449.

3. Julien C, Gorman KF, Akoume MY, Moreau A. Towards a comprehensive diagnostic assay for scoliosis. Personalized Medicine. 2013; 10(1);97-103.

4. Akoume, M. Y., Franco, A., Moreau, A. Cell-Based Assay Protocol for the Prognostic Prediction of Idiopathic Scoliosis Using Cellular Dielectric Spectroscopy. J. Vis. Exp. (2013), e50768, doi: 10.3791/50768.

Skeletal growth, bone health, and bioavailability of leptin in adolescent idiopathic scoliosis

AIS Multidisciplinary Research Team

Team Leader: Jack CY Cheng

Clinicians (Orthopaedics) - Bobby Ng, TP Lam, Alec Hung
Clinician (ENT) - Michael Tong, Waitsz Chang
Clinician (Radiologist) - Winnie Chu, Shi Lin, Defeng Wang
Biochemistry – Gang Li, Kingston Mak, Chao Wan, Wayne Lee
Geneticists - Nelson Tang, TF Chan
Bone biologists - Ling Qin, Louis Cheung, Vivian Hung, Tracy Zhu

Collaborating Partners

Joint Scoliosis Research Centre of The Chinese University of Hong Kong and Nanjing University – Prof. Yong Qiu and colleagues
International collaborations – Montreal, Utrecht, Nottingham, Japan, USA, Australia

Previous studies done in our center have provided strong evidences that AIS was associated with abnormal anthropometric parameters with greater stature, longer arm span and abnormal skeletal development manifested as low aBMD, low vBMD and deranged trabecular bone micro-architecture. Low aBMD and QUS parameters have been shown to be significant prognostic factors for AIS. Along this line of research, AIS was found to be associated with abnormal leptin bioavailability and its correlation with body composition and bone parameters were distinctly different from controls.
The results from these scientific studies spelled out clearly these abnormal skeletal development and its related biochemical anomalies in leptin metabolism could play an important role. Further studies in these areas could potentially pave ways for in-depth understanding of the etiopathogenesis of AIS.

Introduction

The team led by Prof Jack CY Cheng conducted basic and clinical research related to AIS with special emphasis on the etiopathogenesis. The multidisciplinary research team is supported by an established scoliosis clinic receiving annual new referrals of more than 800, a clinical multimedia AIS database of > 16,000, and well-equipped state-of-the-art bone investigation facility and basic science research laboratory centered at Prince of Wales Hospital of the Chinese University of Hong Kong. Many research projects has been conducted, completed and underway with active collaborations with many international centres. The projects related to neuroanatomical changes in the Central Nervous System (including the spinal cord and brain) in AIS: MRI based Study – is covered in another section prepared by Prof Winnie Chu.

Background

Abnormal skeletal growth in AIS has been widely reported to be associated with the development and progression of the scoliotic curves (1, 2). Patients with AIS were found to be taller (3), some studies reported that the stature returns to normal at skeletal maturity (4), while others have reported the increase in height persisted across puberty (3). Although previous studies indicated that there was abnormal skeletal growth in AIS, most of the studies have inadequate sample size, lack in detailed anthropometric measurements, and have inadequate information on pubertal status.

Apart from this abnormal skeletal growth, low bone mineral density (BMD), or osteopenia defined as Z score <-1 was present in 36-38% of AIS girls, and found to be systemic in nature and affecting both peripheral and axial skeletal sites (5). The osteopenia could

persist into adulthood thus leading to osteoporosis and subsequent serious health problems in older age.

Leptin is involved in different physiological processes during puberty. We speculated that leptin could be an important factor that mediates the expression of abnormal phenotypes in AIS including the development of scoliosis. The effects of leptin include suppression of food intake, stimulation of energy expenditure, and regulation of bone metabolism. Leptin-deficient individuals are hypogonadal and may lead to delayed onset of puberty (6, 7). It has been shown that leptin level is also positively correlated with whole body BMD (8). Low level of leptin was associated with increased incidence of osteoporosis in patients with anorexia nervosa (9). The administration of leptin to the leptin-deficiency mice can significantly increase the femoral length (10). These findings related to leptin coincided well with the phenotypes of AIS. In addition, the strength of the leptin signal could also be affected by soluble leptin receptor (sOB-R), its binding protein in circulation. sOB-R could modulate the serum leptin level, and affect bioavailability of the biologically active free leptin (Figure 1). Leptin bioavailability could play an important role in the development of abnormal growth and skeletal development that characterize AIS.

Hypothesis and Methodology

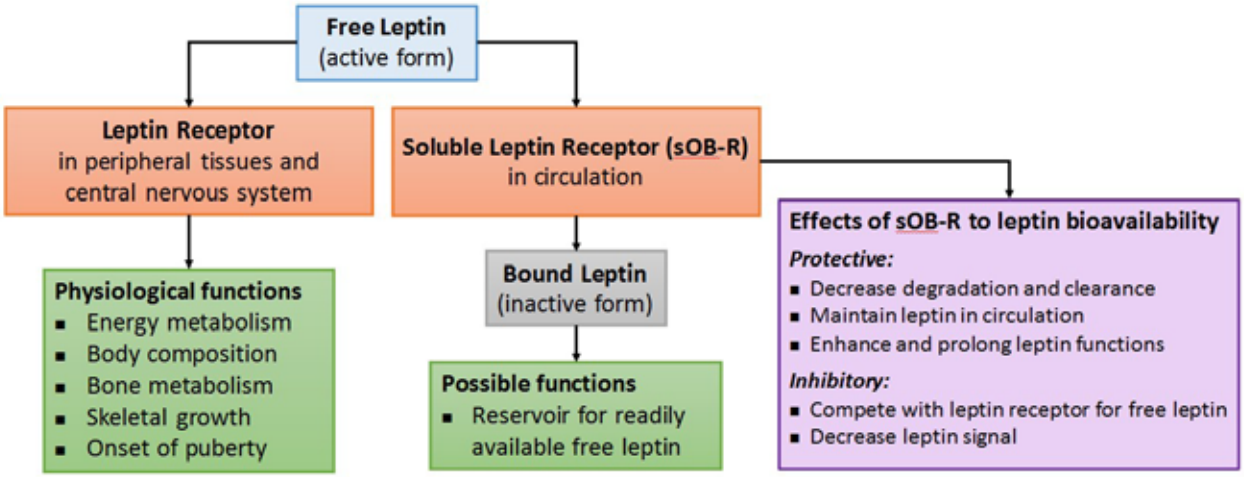


Figure 1. *Free leptin binding and potential roles of soluble leptin receptor (sOB-R) to leptin bioavailability*

We hypothesized that abnormal skeletal growth and suboptimal bone quality are present in AIS and are associated with curve severity and curve progression, and that the bioavailability of free leptin is associated with these abnormal features in AIS girls.

Skeletal growth: Both cross-sectional and longitudinal studies were conducted. In the cross-sectional study, 598 AIS and 307 controls girls were recruited. Weight, height, arm span, sitting height, and leg length were measured. Pubertal development was evaluated according to Tanner's staging. The correlations between anthropometric parameters and curve severity were investigated. In the longitudinal study, 194 AIS and 116 control girls were followed up until skeletal maturity. The AIS girls were grouped into moderate (AIS20) and severe curve (AIS40) groups on the basis of maximum curve magnitude at skeletal maturity. Linear mixed modelling with respect to age or years since menarche was employed to formulate the growth trajectory of different anthropometric parameters.

Bone quality : A series of studies were performed with dual energy X-ray absorptiometry (DXA), peripheral quantitative computed tomography (pQCT), quantitative ultrasound (QUS), high resolution pQCT (HR-pQCT) to evaluate areal BMD (aBMD), volumetric BMD (vBMD), bone morphometry and trabecular bone micro-architecture of axial and peripheral skeletal sites of AIS and control girls. Two large cross-sectional studies with over 600 AIS and 300 control girls were performed to investigate the calcium intake, bone turnover markers, and the aBMD, bone mineral content (BMC) of lumbar spine, proximal femur and distal tibia, and their association with curve severity. aBMD of the spine and both hips measured with DXA, and calcaneus measured with QUS were used to evaluate their prognosticating value in predicting curve progression in two prospective longitudinal studies. In another longitudinal study with 3.5 years follow-up, bilateral femoral neck aBMD and vBMD of the distal tibia were obtained by DXA and pQCT in 196 AIS and 122 control girls to investigate whether osteopenia was a

persistent phenomenon in skeletally matured AIS girls. With more sophisticated equipment which allowed assessment of bone morphometry and trabecular bone micro-architecture, the bone quality of AIS girls was evaluated with HR-pQCT in a cross-sectional study on 214 AIS and 187 controls girls.

Bioavailability of leptin:

This was a 2 year longitudinal cohort study with 179 AIS girls aged 12-14 and 117 age-matched healthy females recruited for baseline comparison, and 95 AIS girls were followed every six months for curve progression and leptin bioavailability. The relationship between leptin bioavailability and curve progression in AIS girls was investigated. Body height, body weight, BMI and Tanner sexual maturity scale were recorded during each visit. The whole body composition was assessed by bioelectric impedance analysis (BIA) to provide the fat and lean mass of the subjects. DXA was used to measure the aBMD of bilateral femoral necks.

Results

Skeletal growth: AIS girls had significantly shorter stature, lower corrected height, and shorter arm span, less sitting height, and shorter leg length than the controls at pubertal stage I. From pubertal stages II through V, corrected height and arm span were significantly greater in the AIS than controls. Corrected sitting height was also greater in AIS from stages II through IV. Furthermore, BMI of AIS was significantly lower than that of controls from stages II through IV. In addition, significant correlations of curve severity with weight, BMI, and arm span were detected. In the longitudinal study, girls with severe AIS were found to have delayed menarche with faster skeletal growth as measured with arm span between 12 – 16 years of age.

Bone quality:

From age 13 years onwards, the AIS group had generalized low bone mass in most axial and peripheral skeletal sites as compared with controls. bALP levels were 38.6% higher in AIS than in controls. A stronger inverse correlation between bALP and bone mass was noted in the AIS group. The bALP was positively correlated with bone area of tibia in the AIS group only. Deoxypyridonine of the AIS group was not different from the controls until age 15 years. The mean calcium intake of the AIS group was very low (only 361 mg/day), and calcium intake was significantly associated with bone mass in the AIS group. Age-adjusted Cobb angle was inversely correlated with BMD and BMC of the distal tibia and lumbar spine among AIS subjects. The proportion of osteopenic AIS girls in the severe group was significantly higher than that in the moderate group. Multivariate analysis showed that Cobb angle was inversely and independently associated with axial and peripheral BMD and BMC. Osteopenia with Z-score <-1 measured by DXA at the femoral neck of the hip on the side of curve concavity (odds ratio = 2.3) and stiffness index measured by QUS (odds ratio = 2.0) were identified as independent and significant prognostic factors for curve progression in AIS. The area under the receiver operating characteristic (ROC) curve with DXA and QUS measurements were 0.80 and 0.83 respectively. Longitudinal study also revealed that over 86% of osteopenic AIS patients had persistently low BMD, at both distal tibia and femoral neck regions, at the time of skeletal maturity. In the HR-pQCT study, we demonstrated that AIS was associated with lower cortical bone area, cortical bone vBMD, trabecular number and greater trabecular separation (Figure 2).

After adjustment for age, dietary calcium intake and physical activity level, the association of AIS with

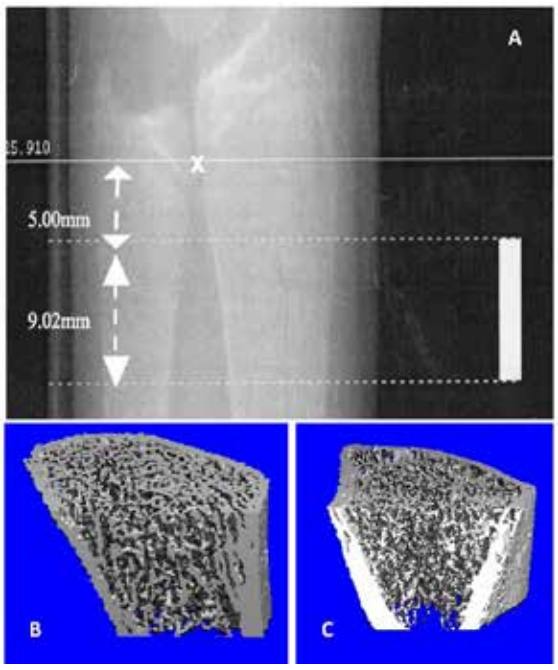


Figure 2. ***(A)*** *Representative scout view of the distal radius scanned with high resolution peripheral quantitative computed tomography (HR-pQCT). The reference line is marked at the most proximal point of the inner aspect of the growth plate (marked with x). The region of interest (ROI) spanning 9 mm starts from 5 mm proximal to the reference line. 110 CT slices with a nominal resolution (voxel size) of 82 ìm were obtained.*

Representative 3D reconstructions of trabecular and cortical bone of distal radius for ***(B)*** *normal control and* ***(C)*** *AIS with osteopenia. Alterations in the trabecular bone micro-architecture could be visualized in AIS with osteopenia.*

lower cortical bone vBMD, lower trabecular number and greater trabecular separation remained statistically significant.

Bioavailability of leptin

AIS girls have significantly higher sOB-R, similar levels of serum total leptin, and lower free leptin index (FLI). The difference remained significant after adjusted for age and body weight. This abnormal leptin bioavailability is associated with lower aBMD at both femoral necks and lower body fat, %body fat, and skeletal muscle mass after adjusted for physical activity level. Serum total leptin and sOB-R were found to correlate with a number of anthropometric parameters such as age, body weight, and BMI. AIS girls lacked the association between serum total leptin and sOB-R. Longitudinal study indicated that AIS patients have increasing serum total leptin and slightly decreasing sOB-R levels during puberty, which agreed with the trend of normal adolescents as reported by Kratzsch et al. in 2002. sOB-R were found to correlate with both baseline and follow-up Cobb angle. FLI correlated with baseline, however the correlation became insignificant with follow-up Cobb angle. Serum total leptin, sOB-R and FLI were not significantly different between the non-progressive and progressive groups of AIS patients.

Summary and Conclusions

Previous studies done in our center have provided strong evidences that AIS was associated with abnormal anthropometric parameters with greater stature, longer arm span and abnormal skeletal development manifested as low aBMD, low vBMD and deranged trabecular bone micro-architecture. Low aBMD and QUS parameters have been shown to be significant prognostic factors for AIS. Along this line of research, AIS was found to be associated with abnormal leptin bioavailability and its correlation with body composition and bone parameters were distinctly different from controls. The results from these scientific studies spelled out clearly abnormal skeletal development and its related biochemical anomalies in leptin metabolism could play an important role. Further studies in these areas could potentially pave ways for in-depth understanding of the etiopathogenesis of AIS.

Selected Publications

1. Cheung, C. S. K., W. T. K. Lee, Y. K. Tse, S. P. Tang, K. M. Lee, X. Guo, L. Qin and J. C. Y. Cheng (2003). «Abnormal peri-pubertal anthropometric measurements and growth pattern in adolescent idiopathic scoliosis: a study of 598 patients.» Spine **28**(18): 2152-2157.

2. Hung, V. W. Y., L. Qin, C. S. K. Cheung, T. P. Lam, B. K. W. Ng, Y. K. Tse, X. Guo, K. M. Lee and J. C. Y. Cheng (2005). «Osteopenia: a new prognostic factor of curve progression in adolescent idiopathic scoliosis.» Journal of Bone and Joint Surgery Am **87**(12): 2709-2716.

3. Lam, T. P., V. W. Hung, H. Y. Yeung, W. C. Chu, B. K. Ng, K. M. Lee, L. Qin and J. C. Cheng (2013). «Quantitative ultrasound for predicting curve progression in adolescent idiopathic scoliosis: a prospective cohort study of 294 cases followed-up beyond skeletal maturity.» Ultrasound Med Biol **39**(3): 381-387.

4. Lam, T. P., V. W. Hung, H. Y. Yeung, Y. K. Tse, W. C. Chu, B. K. Ng, K. M. Lee, L. Qin and J. C. Cheng (2011). «Abnormal bone quality in adolescent idiopathic scoliosis: a case-control study on 635 subjects and 269 normal controls with bone densitometry and quantitative ultrasound.» Spine (Phila Pa 1976) **36**(15): 1211-1217.

5. Lee, W. T. K., C. S. K. Cheung, Y. K. Tse, X. Guo, L. Qin, S. C. Ho, J. Lau and J. C. Y. Cheng (2005). «Generalized low bone mass of girls with adolescent idiopathic scoliosis is related to inadequate calcium intake and weight bearing physical activity in peripubertal period.» Osteoporos Int **16**: 1024-1035.

6. Lee, W. T. K., C. S. K. Cheung, Y. K. Tse, X. Guo, L. Qin, T. P. Lam, B. K. W. Ng and J. C. Y. Cheng (2005). «Association of osteopenia with curve severity in adolescent idiopathic scoliosis: a study of 919 girls.» Osteoporos Int **16**: 1924-1932.

7. Yim, A. P., H. Y. Yeung, V. W. Hung, K. M. Lee, T. P. Lam, B. K. Ng, Y. Qiu and J. C. Cheng (2012). «Abnormal skeletal growth patterns in adolescent idiopathic scoliosis—a longitudinal study until skeletal maturity.» Spine (Phila Pa 1976) **37**(18): E1148-1154.

8. Yu, W. S., K. Y. Chan, F. W. Yu, B. K. Ng, K. M. Lee, L. Qin, T. P. Lam and J. C. Cheng (2014). «Bone structural and mechanical indices in Adolescent Idiopathic Scoliosis evaluated by high-resolution peripheral quantitative computed tomography (HR-pQCT).» Bone **61C**: 109-115.

9. Liu, Z., E. M. Tam, G. Q. Sun, T. P. Lam, Z. Z. Zhu, X. Sun, K. M. Lee, T. B. Ng, Y. Qiu, J. C. Cheng and H. Y. Yeung (2012). Abnormal leptin bioavailability in adolescent idiopathic scoliosis: an important new finding. Spine (Phila Pa 1976) 37(7): 599-604.

10. Tam, E. M., F. W. Yu, V. W. Hung, Z. Liu, K. L. Liu, B. K. Ng, S. K. Lee, Y. Qiu, J. C. Cheng and T. P. Lam (2014). Are volumetric bone mineral density and bone micro-architecture associated with leptin and soluble leptin receptor levels in adolescent idiopathic scoliosis? - a case-control study. PLoS One 9(2): e87939.

Sources of References

1. Porter RW. Idiopathic scoliosis: The relation between the vertebral canal and the vertebral bodies. Spine. 2000;25(11):1360-6.

2. Burwell RG, Dangerfield PH, Freeman BJ. Etiologic theories of idiopathic scoliosis. Somatic nervous system and the NOTOM escalator concept as one component in the pathogenesis of adolescent idiopathic scoliosis. Stud Health Technol Inform. 2008;140:208-17.

3. Willner S. The proportion of legs to trunk in girls with idiopathic structural scoliosis. Acta Orthopaedica Scandinavica. 1975;46(1):84-9.

4. Goldberg CJ, Dowling FE, Fogarty EE. Adolescent idiopathic scoliosis - Early menarche, normal growth. Spine. 1993;18(5):529-35.

5. Cheng JCY, Qin L, Cheung CSK, Sher AHL, Lee KM, Ng SWE, et al. Generalized low areal and volumetric bone mineral density in adolescent idiopathic scoliosis. Journal of Bone and Mineral Research. 2000;15(8):1587-95.

6. Farooqi IS. Leptin and the onset of puberty: insights from rodent and human genetics. Semin Reprod Med. 2002;20(2):139-44.

7. Farooqi IS, Matarese G, Lord GM, Keogh JM, Lawrence E, Agwu C, et al. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. J Clin Invest. 2002;110(8):1093-103.

8. Matkovic V, Ilich JZ, Skugor M, Badenhop NE, Goel P, Clairmont A, et al. Leptin is inversely related to age at menarche in human females. J Clin Endocrinol Metab. 1997;82(10):3239-45.

9. Baldock PA, Sainsbury A, Allison S, Lin EJ, Couzens M, Boey D, et al. Hypothalamic control of bone formation: distinct actions of leptin and y2 receptor pathways. J Bone Miner Res. 2005;20(10):1851-7.

10. Iwaniec UT, Boghossian S, Lapke PD, Turner RT, Kalra SP. Central leptin gene therapy corrects skeletal abnormalities in leptin-deficient ob/ob mice. Peptides. 2007;28(5):1012-9.

The relationship of curve laterality and melatonin contents in cerebral hemisphere in melatonin-deficient scoliotic mice

Professor Masafumi Machida,M.D.

Project description

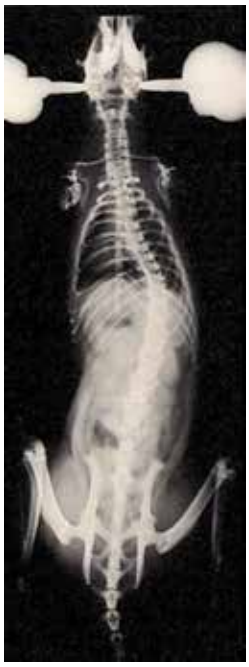
This research project is designed to investigate the curve laterality of adolescent idiopathic scoliosis (AIS) in melatonin-deficient C57BL/6J mice that develop scoliosis. Scoliosis in these bipedal mice is induced without pinealectomy, and melatonin administration suppresses the development of scoliosis.

This suggests that melatonin deficiency plays a crucial role in the development of scoliosis. In these mice, SEPs after leg stimulation were abnormal or absent in the scoliosis group compared to the non-scoliosis group with melatonin administration. The type of SEPs abnormality suggests a lesion from the brainstem to cortex, strongly supporting our hypothesis that AIS results from dysfunction of the central nervous system. However, the mechanism behind curve laterality in AIS remains unknown.
Cerebral function asymmetry has been considered a unique characteristic of the human brain. In the last several years, neuroanatomical, biochemical, and functional evidence of asymmetry has been demonstrated in the brains of various animal species.

Scoliosis has been reported in animals with experimentally induced hemiparkinsonism with thoracic-lumbar curves oriented towards the lesion side and its severity directly associated with the degree of striatal dopamine decrease. The scoliosis is speculated to reflect the functional asymmetry of dopamine activity in the basal ganglia where the spine concaves to the side with dopamine hypoactivity. In fact, postencephalitic hemiparkinsonism subjects, develop scoliosis with concavity ipsilateral to the affected substantia nigra. This scoliotic deformity can be considered to be similar to that found in our previous studies on scoliosis and AIS.

In our previous study of chickens, significant asymmetries of melatonin content were found in each hemisphere, both in normal animals and in pinealectomized chickens with scoliotic deformity. This indicates that hemispheric asymmetry in chickens is more prevalent, as well as more complex, than previously thought.

In this project we have assessed potential asymmetries by measuring the local rates of melatonin utilization in several region of the mouse brain. Asymmetrical change of melatonin contents in the cerebral hemisphere supports structures, growth centers, the position of spine, and neural or muscular components can result in the development of scoliosis. It now appears that different asymmetries are organized along different dimensions in mouse and the human brains.



Scoliotic deformity with vertebral rotation in pinealectomized bipedal rat. Machida M et al. Spine 1999;24:1986.

CHAPTER 3

Neurosensorial abnormalities

The Neurosensorial studies explore the balance of the body, the perception of the body in space, the asymmetry between the right and left cerebral hemisphere and the processing and integration of this information by the Central Nervous system.
Many international teams (in France, Canada and Hong Kong) work on the hypothesis that the Adolescent idiopathic Scoliosis could be associated with disturbances of the propRioceptive perception (illustrated by anatomic and functional abnormalities: asymmetry of the skull, brain or vestibular organs, etc) and its integration in the Central Nervous system.
The development of new imaging techniques as the diffusion tensor imaging (DTI), the creation of animal models and easier tests to study the functional aspect of the vestibular system have opened new and promising perspectives.

Neural Basis of Proprioceptive Processing in Adolescent Idiopathic Scoliosis

Christine ASSAIANTE
Marianne VAUGOYEAU
Fabien CIGNETTI
Laboratoire de Neurosciences Cognitives UMR 7291, CNRS-AMU, Marseille
Both neurosensory tests and fMRI studies will be performed in Timone Hospital in Marseille, in close cooperation with the Orthopedic Infantile Unit, directed by
Professor Jean-Luc Jouve and **Professor Gérard Bollini**

This project is based on the developmental expertize of our research group which associates functional approach in the field of sensorimotor control with studies of brain structures involved in perception-action coupling, body schema and internal models of action in children and adolescents with typical or atypical neurodevelopment.

Proprioception has been found critical for controlling multi-joint movements and for establishing internal models of limb representation (body schema) used in learning and adaptation of action. Given this importance, several studies have examined the development of the proprioceptive ability and found that it improves throughout childhood and well into adolescence.There is no doubt that such improvement is subtended by maturation of the central proprioceptive network during the key period of adolescence. Our findings reveal plasticity (i.e., refinement) of the proprioceptive network during adolescence. In particular, this refinement is consistent with the viewpoint of a hierarchical sequence of functional development from sensorimotor to association cortex. A decreased brain activation in primary somatosensory cortex in AIS would support the view that a sensorimotor integration disorder may underlay the pathogenesis of IS.

Background

Idiopathic scoliosis is a developmental pathology which expresses spinal deformity mainly during adolescence, a period of physiological and psychological transition, which is known to involve considerable morphological, structural and functional changes (Morris & Udry, 1980; Rogol *et al.*, 2002).
Neuroscience research has made important contributions to our understanding of development by demonstrating that the brain is far more plastic at all ages than previously thought and the remarkable role of experience in shaping the mind, brain and body (Diamond and Amso, 2008).
The question arises as to what effects these huge body changes including spinal deformity may have on AIS' body scheme, which serves as a reference frame to control posture and its vertical representation, action and representation of action. Herman et al (1985) as well as Burwell and colleagues (2008) indicate that maturation of CNS and building of body scheme are relevant components of idiopathic scoliosis. Up to now, a functional magnetic resonance imaging (fMRI) study aiming at building a link between the sensory deficits previously evidenced in AIS, and a central deficit of multisensory integration in the course of the ontogenesis has not been investigated.

Hypothesis

Our main goal is to study how development interferes with pathology in case of adolescents suffering spinal deformities. Although the aetiology and pathophysiology of adolescent idiopathic scoliosis (AIS) is still not well understood, an increasing number of studies suggests that deficits in sensory integration could contribute to the cause of IS. In particular, proprioceptive processing may be atypical in AIS, leading to inappropriate motor commands and balance control problems. We propose to record brain activity using fMRI with AIS and healthy adolescent controls experiencing kinaesthetic illusions evoked by tendon vibrations. Our hypothesis is that a disturbed proprioceptive integration might be correlated with abnormal changes of activity in some of the areas and networks involved in the illusion task. The comparison with a healthy adult group will be necessary to dissociate the developmental brain mechanisms.

The subjects will undergo a muscle tendon vibration protocol, supine into a 3-Tesla fMRI scanner. Pneumatic vibration devices will be placed on the right and left tendons of the tibialis anterior muscles, providing low- (30 Hz) and high-frequency (100 Hz) stimulations. These parameters are selected because 30 Hz stimulation drives weak discharges of the primary endings and 100 Hz frequency optimally activates primary endings, so that contrasting the two conditions will reveal the base network of proprioception-related activity. fMRI time series will be analysed using general linear models and region of interest analyses.

Results

54 subjects took part into the experiment, subdivided into three groups (18 early adolescents: 10-14 years; 18 adolescents: 14-18 years; 18 adults). Our results revealed that adults and adolescents activated a similar distributed network of primary sensorimotor regions and higher-order association (i.e., frontoparietal) regions. The main developmental effect that emerges from this study is on functional connectivity. The number and the strength of brain regions functionally interacting within the proprioceptive network decrease with age (see Fig 1). This finding reflects a process of over-connectivity followed by pruning with age, which restructures connectivity of the proprioceptive network in the developing brain. The way these functional changes at the level of the brain (i) are related to structural features, such as grey/white matter density and maturation of white matter tracts, and (ii) differ in a population of AIS is currently under examination.

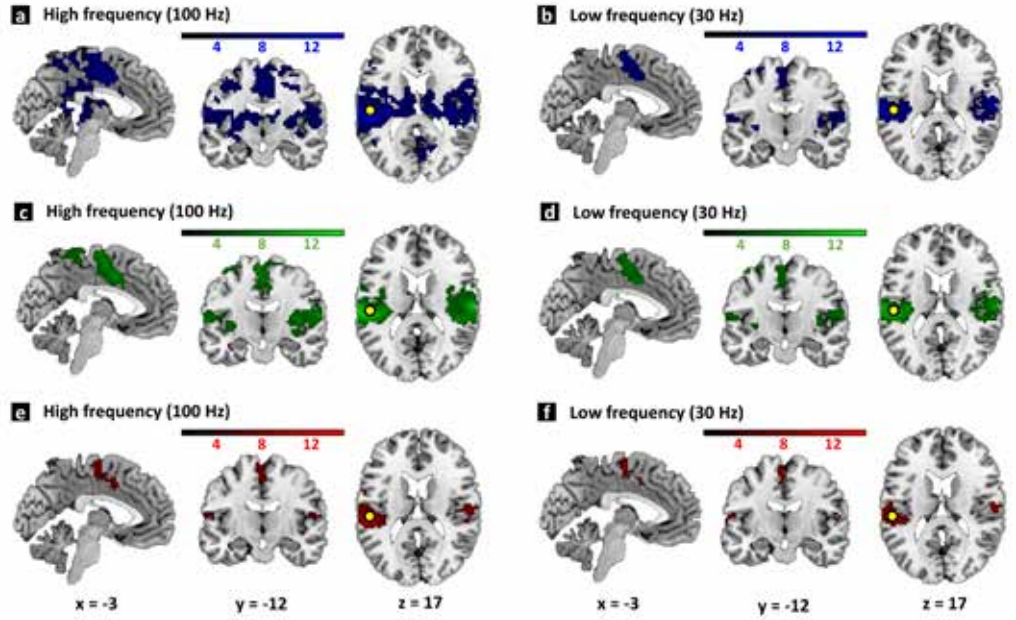


Figure 1: *Diffuse-to-focal shift in the pattern of functional connectivity (FC) as a function of age group (early adolescents are depicted in blue, adolescents in green and young adults in red). Maps 1a, 1c and 1e illustrate FC during high-frequency proprioceptive stimulations while maps 1b, 1d and 1f illustrate FC during low-frequency stimulations. A seed-based approach has been used to derive all FC maps, using a region of interest located in the left supramarginal gyrus (represented as a yellow circle).*

Sources

Diamond, A., Amso, D. Contributions of Neuroscience to Our Understanding of Cognitive Development. (2008). Current Directions in Psycholol Science, 17, 136-141

Herman, R., Mixon, J., Fisher, A., Maulucci, R., Stuyck, J. Idiopathic scolosis and the central nervous system: a motor control problem. (1985) The Harrington lecture, 1983. Scoliosis Research Society. Spine 10: 1-14.

Morris, NM & Udry, JR Validation of a self administered instrument to assess stage of adolescent development. (1980). Journal of Youth and Adolescence, 9, 271-280.

Rogol, A.D., Roemmich, J.N., & Clark, P.A. (2002). Growth at puberty. The Journal of adolescent health, 31, 192-200.

Burwell, RG., Dangerfield, PH., Freeman, BJ. Ethiologic theories of idiopathic scoliosis. Somatic nervous system and the NOTOM escalator concept as one component in the pathogenesis of AIS. (2008), Stud Health Technol Inform, 140: 208-17.

Publication

Recent Papers from our group relative to sensorimotor integration during development

Assaiante, C., Mallau, S., Jouve, J.L., Bollini, G., Vaugoyeau, M. (2012) Do adolescent idiopathic scoliosis neglect proprioceptive information in sensory integration of postural control ? PLoS ONE, 7(7):e40646. OI:10.1371/journal.pone.0040646.

Cignetti, F.**,** Chabeauti, P-Y., Sveistrup, H., Vaugoyeau, M., **Assaiante, C.** (2013) Updating process of internal model of action as assessed from motor and postural strategies in children. Neuroscience 2013;233:127-38.

Cignetti, F., Caudron, S., Vaugoyeau, M., **Assaiante, C.** (2013) Body schema disturbance in adolescence: from proprioceptive integration to the perception of human movement. Journal of Motor Learning and Development 2013; 1: 49-58.

Assaiante, C., Barlaam, F., Cignetti, F., Vaugoyeau M. (2014) Building of body schema during childhood and adolescence : a neurosensory approach. Neurophysiologie Clinique / Clinical Neurophysiology, (2014) 44, 3-12.

Cignetti, F., Vaugoyeau, M., Nazarian, B., Roth, M., Anton, JL., **Assaiante, C.** (2014) Boosted activation of right inferior frontoparietal network: a basis for illusory movement awareness. Human Brain Mapping, in press.

What is the association between abnormal anatomy or function of vestibular system with curve progression in Adolescent Idiopathic Scoliosis (AIS)?

Prof Winnie Chu - Professor, Imaging & Interventional Radiology
Prof Jack Cheng- Professor, Orthopaedics and Traumatology
Prof Michael Tong- Professor, Otorhinolaryngology
Institution: Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong SAR, China

Background

The occurrence of abnormal postural control and vestibular-related dysfunction in AIS has been regularly reported (1-4). A number of studies have shown abnormal nystagmus response to caloric testing in patients with AIS, suggesting a defect in the oculo-vestibular system (5-7). Recently, Mallau (8) found the biomechanical defect in AIS mainly affects the yaw head stabilization during locomotion.The change in yaw head stabilization strategy is likely caused by vestibular deficit, a finding that is consistent with studies by Sahlstrand (5) and Yamamoto (9) who also noted the presence of sensory asymmetries in the semicircular canals. Wiener-Vacher and Mazda(10) have reported central otolith vestibular system imbalance in AIS. Changes in the vestibule-ocular reflex in AIS was observed by Herman(11). In addition, Gauchard (4) reported that dynamic balance control was more severely affected in patients with upper scoliotic curvature, since they were less able to reposition their heads horizontally, and this gave rise to vestibular asymmetries. Rousie et al reported abnormal connection between lateral and posterior semicircular canal as revealed by new modeling processes (12).

In an animal model by Lambert(13), a possible vestibular origin was tested in the frog *Xenopus laevis* by unilateral removal of the labyrinthine end-organs at larval stages.After metamorphosis into young adult frogs, x-ray images and 3D reconstructed micro-CT of the skeleton showed deformations similar to those of scoliotic patients. It is proposed that the *Xenopus* model links with human scoliosis because a comparable situation occurs during gestation *in utero*. A permanently imbalanced activity in descending locomotor/ posture control pathways might be the common origin for the observed structural and behavioral deficits in humans as in the different animal models of scoliosis.

There have been many speculations on possible sites of vestibular abnormality in AIS in the past, but to-date, few of its secrets have been surrendered to investigators due to lack of technology to test the hypothesis. Studies of post mortem specimens shows that the radius of the horizontal semicircular canal is of mean 3.17mm, with sd 0.21mm (14). Nowadays, these dedicated human structures can be visualized by high resolution MRI. Morphological changes in semicircular canals, vestibular utricle & saccule and cochlea can now be detected and analysed using our modern advanced computational morphometric analysis technique on MR images.

Hypothesis : We hypothesize that in AIS, abnormal postural balance mechanism could be the result of morphoanatomical and pathophysiological changes in the vestibular system.

Project Objectives

1. To study the morphoanatomical difference of the whole vestibular system between AIS girls with matched controls in a cross-sectional study using validated and in-house developed advanced imaging processing and model-based segmentation techniques that could

accurately delineate the surface anatomy of the semicircular canals, vestibular utricle & saccule and cochlea from high resolution T2-weighted MRI data.

2. To correlate the morphoanatomical variations in the vestibular system in AIS subjects with clinical assessment of vestibular function.
3. To establish whether vestibular dysfunction (morphological and/ or functional) is associated with curve progression in a 2-year longitudinal study

Methodology :

Part I : Cross sectional Study

MRI of the vestibular system and the brain were carried out in untreated AIS subjects newly referred to the Scoliosis Clinic with documented clinical and radiological idiopathic right thoracic curves. The controls included age and sex-matched girls. Modern computational morphometric techniques were used to document the morphometry of semicircular canals, vestibular utricle, saccule, and cochlea as well as segmentation of the brain. Comprehensive standardized physiological tests were performed to assess vestibular function. All the anatomical and functional data of the vestibular system were correlated in both groups while Cobb angle and curve type in AIS girls entered into the regression analysis.

Part II : Longitudinal Study:

AIS girls entered into the study were followed up and evaluated over a two year period. Age and sex-matched normal subjects recruited from the above cross-sectional study entered the longitudinal study and act as controls for analyzing the confounding effect of normal growth on brain and spine. Measurements were made as for the cross-sectional study. The major aims of the longitudinal study are:

(1) To establish whether morphological vestibular abnormalities are associated with curve progression.

(2) To test whether a clinical vestibular functional disorder is associated with curve progression and morphological changes in semicircular canals, vestibular utricle & saccule, and cochlea.

The data from (1) & (2) will be evaluated to establish association and predictive factors for curve progression.

Results: In the first cross-sectional study of 19 AIS girls and 12 matched controls, greater orientation asymmetry has been observed between the right and left vestibular system (VS) along z-axis. There is also statistically significant difference in the shape analysis of the left-side VS between AIS and normal controls, in which firstly the distance between the centers of the lateral and superior canals was smaller in AIS. Secondly, the angle between the center-joining lines at the posterior canal is also smaller in AIS (15).

In the later longitudinal study, we have compared 72 AIS with major right-sided thoracic curve, in which 42 have progressive curve (P-AIS), 26 have non-progressive curve (NP-AIS) and 28 normal controls (NC). The orientation asymmetry and difference in shape of the left-sided VS are more exaggerated in the P-AIS group, followed by the NP-AIS group when compared with NC.

Such a difference; however does not exist in the right-side VS.

The physiological vestibular functional tests including electonystagmography (ENG), vestibular evoked myogenic potential test (cVEMP) and sensory organization test (SOT) however, do not reveal any significant difference between AIS and NC.

In three other related studies involving MR imaging of the brain in AIS, the cerebral cortical thickness maturation pattern, focal cortical thickness and regional cerebellar volumes are found to be significantly different in AIS while cortical structural network pattern in the AIS brain is also altered when compared with age-matched controls. Of note, the above brain regions which show morphological difference between AIS and controls, are related to vestibular/ balance function in human (16-18).

Conclusion and significance

There is evidence of abnormal morphoanatomical changes in the vestibular system in AIS, affecting only the left vestibular system in AIS girls with predominantly right thoracic curve. Such differences are even more prominent in AIS with progressive curve than those with non-progressive curve. This asymmetry in vestibular input probably results in an unbalanced vestibulospinal control and may contribute to the development and progression of scoliotic curve in AIS. The above vestibular abnormalities may or may not be detected by physiological functional tests in AIS subjects, as compensatory adaption from the central nerve system might already be developed in these scoliosis girls but varies from case to case. Such an abnormality however, can be identified by imaging as the formation of vestibular system is completed by birth. The anatomical variations of vestibular system in AIS might help scientists to understand why curve progression occurs in some AIS girls but not all.

Sources and references

1. Byl NN, Holland S, Jurek A, Hu SS. Postural imbalance and vibratory sensitivity in patients with idiopathic scoliosis: implications for treatment. J Orthop Sports Phys Ther. 1997;26(2):60-8.

2. Byl NN, Gray JM. Complex balance reactions in different sensory conditions: adolescents with and without idiopathic scoliosis. J Orthop Res. 1993;11(2):215-27.

3. Adler N, Bleck EE, Rinsky LA, Young W. Balance reactions and eye-hand coordination in idiopathic scoliosis. Journal of Orthopaedic Research. 1986;4(1):102-7.

4. Gauchard GC, Lascombes P, Kuhnast M, Perrin PP. Influence of different types of progressive idiopathic scoliosis on static and dynamic postural control. Spine. 2001;26(9):1052-8.

5. Sahlstrand T, Petruson B. A study of labyrinthine function in patients with adolescent idiopathic scoliosis. I. An electro-nystagmographic study. Acta orthopaedica Scandinavica. 1979;50(6 Pt 2):759-69.

6. O'Beirne J, Goldberg C, Dowling FE, Fogarty EE. Equilibrial dysfunction in scoliosis—cause or effect? Journal of Spinal Disorders. 1989;2(3):184-9.

7. Yamada K, Yamamoto H, Nakagawa Y, Tezuka A, Tamura T, Kawata S. Etiology of idiopathic scoliosis. Clinical Orthopaedics and Related Research. 1984(184):50-7.

8. Mallau S, Bollini G, Jouve JL, Assaiante C. Locomotor skills and balance strategies in adolescents idiopathic scoliosis. Spine. 2007;32(1):E14-22.

9. Yamamoto H, Tani T, MacEwen GD, Herman R. An evaluation of brainstem function as a prognostication of early idiopathic scoliosis. Journal of pediatric orthopedics. 1982;2(5):521-8.

10. Wiener-Vacher SR, Mazda K. Asymmetric otolith vestibulo-ocular responses in children with idiopathic scoliosis. The Journal of pediatrics. 1998;132(6):1028-32.

11. Herman R, MacEwen G, Zorab P, editors. Idiopathic scoliosis: a visio-vestibular disorder of the central nervous system: Academic Press; 1979.

12. Rousie DL, Deroubaix JP, Joly O, Baudrillard JC, Berthoz A. Abnormal connection between lateral and posterior semicircular canal revealed by a new modeling process: origin and physiological consequences. Annals of the New York Academy of Sciences. 2009;1164:455-7.

13. Lambert FM, Malinvaud D, Glaunes J, Bergot C, Straka H, Vidal PP. Vestibular asymmetry as the cause of idiopathic scoliosis: a possible answer from Xenopus. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2009;29(40):12477-83. Epub 2009/10/09.

14. Curthoys IS, Oman CM. Dimensions of the horizontal semicircular duct, ampulla and utricle in the human. Acta oto-laryngologica. 1987;103(3-4):254-61.

15. Shi L, Wang D, Chu WC, Burwell GR, Wong TT, Heng PA, et al. Automatic MRI segmentation and morphoanatomy analysis of the vestibular system in adolescent idiopathic scoliosis. NeuroImage. 2011;54 Suppl 1:S180-8. Epub 2010/04/13.

16. Wang D, Shi L, Chu WC, Burwell RG, Cheng JC, Ahuja AT. Abnormal cerebral cortical thinning pattern in adolescent girls with idiopathic scoliosis. NeuroImage. 2012;59(2):935-42. Epub 2011/08/30.

Neuroanatomical changes in the central nervous system (including the spinal cord and brain) in adolescent idiopathic scoliosis: MRI based Study

Prof Winnie Chu- Professor, Imaging & Interventional Radiology
Prof Jack Cheng- Professor, Orthopaedics and Traumatology
Prof Shi Lin, Assistant Professor, Internal Medicine & Therapeutics
Prof Defeng Wang, Assistant Professor, Imaging & Interventional Radiology

Institution: Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong SAR, China

Using MRI, abnormal morphoanatomical changes are found in the spinal cord, brain and vestibular system in AIS, when compared with age-matched normal controls.
In the spinal cord, there is evidence of cord tethering with relative shortening of the cord, change in cross-sectional shape and position of the cord, low-lying cerebellar tonsils and microstructural change within the cord with reduced diffusivity.
In the brain, structural changes of certain parts of the brain are found including the corpus callosum. These areas are related to either motor control, vestibular or somatosensory functions.
In the vestibular system, morphological difference is found in the left semicircular canals of AIS girls with predominantly right thoracic curve. Such difference is more prominent in patients with progressive curve.
It is not yet certain whether the above changes in the CNS is primary (i.e. causing the scoliosis) or secondary (i.e. due to adaption/ compensation to the scoliosis); however the MRI studies prove that in AIS, not only the spine is affected but also the CNS. The findings are in agreement with a number of well documented functional neurological abnormalities which have been reported in literature.

Background

Previously people thought scoliosis only affected the spine, and the vertebrae that make it up. The cause of the disease had previously been a mystery, although the fact that it recurs in certain families, and targets females in particular, suggests there's a large genetic component. It's also possible that, since girls are more slender and often taller than boys in adolescence, it is a by-product of growing spurts that leave the body unable to handle the rapid growth of the spine. The spinal deformity is simply the most visible symptom of the disease, while there are numerous «hidden» features that have yet to be fully explored.

Recently more evidence suggests that scoliosis is a systemic disorder, which does not only affect the central spine, but also the appendiceal skeleton, and the overall bone density [1-3]. The concept of asynchronous growth between the neural and skeletal systems was first proposed by Roth [4] and rediscovered by Porter [5, 6]. According to this model, when spurts of elongation of the spine are too rapid for the slower growth rate of the spinal cord and nerve roots, the neurovertebral growth disproportion is compensated for by adaptive scoliotic curvature of the otherwise normally growing spine.

The nervous system is also likely to be involved in causing curvature of the spine. Abnormal

somatosensory function has been widely reported in this group, including prolonged latency or absent waveforms in posterior tibial nerve somatosensory evoked potential in different series[7]. The abnormal balance control in AIS may be affected by somatosensory input[8]. A number of studies have shown an abnormal nystagmus response to caloric testing in patients with AIS, suggesting a defect in the oculo-vestibular system [9-11]. Asymmetries of neurological function are more pronounced in children with AIS [12-15] and are being attributed to the cerebral cortex and central programming[16, 17]. Goldberg [18] also showed the organization of the brain for dichotic listening was more strongly lateralized in AIS than non-scoliosis subjects. Burwell and Dangerfield [19, 20] have suggested the neuro-osseous timing of maturation (NOTOM) concept about CNS postural maturation, which explains why girls are more susceptible than boys in developing scoliosis, as girls enter their adolescent growth spurt earlier, before their postural mechanism are mature.

By studying the parts of the brain that are concerned with how the body balances itself, an asymmetry in those systems that potentially could throw them off balance might also be disclosed.
X-ray technology has yielded images of the spinal structure for more than a century. But it examined just the bones and the shape of the spine. It's only through advances in magnetic resonance imaging, or MRIs, that it has become possible to scan the activity of the nervous system and spinal column in any greater detail. Recently, with advances in technology, morphological differences in the CNS, including both the brain and spinal cord, are beautifully demonstrated using magnetic resonance imaging (MRI) equipped with advanced imaging sequences and computational technique.

Hypothesis: In AIS girls, when spurts of elongation of the spine are too rapid for the slower growth rate of the spinal cord and nerve roots, the neurovertebral growth disproportion is compensated for by adaptive scoliotic curvature of the otherwise normally growing spine.

Part I : cross sectional

MRI of the brain and radiological examination of the spine will be carried out in 100 untreated AIS subjects including right thoracic curves. The controls are 100 age and sex matched girls. Modern morphometric techniques of brain analysis to determine regional brain volume differences and brain asymmetries will be applied. In AIS girls, associations for spine and brain parameters will be evaluated with anthropometric measurements, routine clinical neurological tests, SSEPs, postural balance tests, menarche, Tanner sexual maturity stage, Risser sign, tri-radiate cartilage and phalangeal epiphyses

Part II : longitudinal

100 of the above AIS girls will be evaluated over two years. Bracing history will be recorded. 50 of age and sex-matched normal subjects recruited from the above cross-sectional study will also enter the longitudinal study and act as controls for consideration of the confounding effect of normal growth on brain and spine. Measurements will be made as for the cross-sectional study.

The major aims of the longitudinal study are as follows:

(1) To establish whether brain abnormalities are associated with curve progression. Findings of neuro-functional tests are also correlated.

(2) To test whether each of (a) cord-to-vertebral length ratio and (b) apical cord shape change are associated with curve progression and focal changes in the brain.
The data from (1) & (2) will be evaluated to establish association and predictive factors for curve progression.

Results

In the study of the spine and spinal cord, AIS has been found to have increased total vertebral column length predominantly affecting the thoracic segment, without corresponding lengthening of the spinal cord (1), which is associated with distortion of cross-sectional shape of the cord at the level of scoliotic ápex (2) and resulting low- lying cerebellar tonsil (3). This reduced spinal cord to vertebral column ratio supports the hypothesis by Roth and Porter that a short, unforgiving spinal cord could produce the abnormal rotator anatomy observed at the apex in scoliosis, which gives rise to lordosis, predisposing to lateral deviation and rotatory

deformity of the vertebral column as in AIS. Porter has suggested that the spinal cord might fail to stretch in response to vertebral growth due to molecular mechanism. The damage to the white matter tract within the spinal cord has been recently demonstrated by a study using advanced diffusion tensor imaging (DTI) technique, in which significantly decreased fractional anisotropy (FA) values and increased mean diffusivity (MD) values are found at the medulla oblongata and upper cervical segment (C1-C5) of the spinal cord in AIS when compared with normal controls (4). This microstructural change in the neural pathway is in agreement and probably accounts for the clinical observation of abnormal somatosensory evoked potential (SEP) commonly found in AIS.

In the study of brain, the mean brain volume after normalization has been found to be significantly different in 22 of 99 anatomical regions (5) in AIS subjects, including corpus callosum and brainstem. A subsequent study analyzing 2D shape has shown that the splenium of corpus callosum (a region known to connect the motor and premotor cortices of the two hemispheres) is consistently different from normal controls (6). The above observation leads to the hypothesis that a primary deficit in interhemispheric co-ordination might play a primary role in the etiopathogenesis of AIS. In other studies, the cerebral cortical thickness maturation pattern, focal cortical thickness (7) and regional cerebellar volumen (8) are found to be significantly different in AIS while cortical structural network pattern in the AIS brain is also altered when compared with age-matched controls (9). Of note, part of the brain which shows morphological difference between AIS and controls, is related to either motor control, vestibular or somatosensory functions. Interestingly, in a morphological study of vestibular system in AIS, greater orientation asymmetry has been observed between the right and left vestibular system (VS) along z-axis. There is also statistically significant difference in the shape analysis of the left-side VS between AIS and normal controls (10). It is not yet certain whether the above brain changes are primary (i.e. etiopathogenetic) or secondary (i.e. adaptation/ compensation) to the development of scoliosis; however the anatomical brain changes correspond with a number of well-documented functional neurological abnormalities as described above.

Sources and references.

1. Cheng, J.C. & Guo, X. Osteopenia in adolescent idiopathic scoliosis. A primary problem or secondary to the spinal deformity? Spine (Phila Pa 1976) **22**, 1716-21 (1997).
2. Cheng, J.C. et al. Persistent osteopenia in adolescent idiopathic scoliosis—longitudinal monitoring of bone mineral density until skeletal maturity. Stud Health Technol Inform **123**, 47-51 (2006).
3. Cheung, C.S. et al. Generalized osteopenia in adolescent idiopathic scoliosis—association with abnormal pubertal growth, bone turnover, and calcium intake? Spine (Phila Pa 1976) **31**, 330-8 (2006).
4. Roth, M. Idiopathic scoliosis caused by a short spinal cord. Acta Radiol Diagn (Stockh) **7**, 257-71 (1968).
5. Porter, R.W. Idiopathic scoliosis: the relation between the vertebral canal and the vertebral bodies. Spine (Phila Pa 1976) **25**, 1360-6 (2000).
6. Porter, R.W. The pathogenesis of idiopathic scoliosis: uncoupled neuro-osseous growth? Eur Spine J **10**, 473-81 (2001).
7. Cheng, J.C., Guo, X. & Sher, A.H. Posterior tibial nerve somatosensory cortical evoked potentials in adolescent idiopathic scoliosis. Spine (Phila Pa 1976) **23**, 332-7 (1998).
8. Guo, X. et al. Balance control in adolescents with idiopathic scoliosis and disturbed somatosensory function. Spine (Phila Pa 1976) **31**, E437-40 (2006).
9. Sahlstrand, T. & Petruson, B. A study of labyrinthine function in patients with adolescent idiopathic scoliosis. I. An electro-nystagmographic study. Acta Orthopaedica Scandinavica **50**, 759-769 (1979).
10. O'Beirne, J., Goldberg, C., Dowling, F.E. & Fogarty, E.E. Equilibrial dysfunction in scoliosis—cause or effect? Journal of Spinal Disorders **2**, 184-189 (1989).
11. Yamada, K. et al. Etiology of idiopathic scoliosis. Clinical Orthopaedics and Related Research, 50-57 (1984).
12. Barrack, R.L., Whitecloud, T.S., III, Burke, S.W., Cook, S.D. & Harding, A.F. Proprioception in idiopathic scoliosis. Spine **9**, 681-685 (1984).
13. Barrack, R.L. et al. Vibratory hypersensitivity in idiopathic scoliosis. Journal of Pediatric Orthopedics **8**, 389-395 (1988).

14. Cook, S.D. et al. Upper extremity proprioception in idiopathic scoliosis. Clinical Orthopaedics and Related Research, 118-124 (1986).
15. Wyatt, M.P., Barrack, R.L., Mubarak, S.J., Whitecloud, T.S. & Burke, S.W. Vibratory response in idiopathic scoliosis. Journal of Bone and Joint Surgery. British Volume **68**, 714-718 (1986).
16. Maguire, J., Madigan, R., Wallace, S., Leppanen, R. & Draper, V. Intraoperative long-latency reflex activity in idiopathic scoliosis demonstrates abnormal central processing. A possible cause of idiopathic scoliosis. Spine **18**, 1621-6 (1993).
17. Brinker, M.R. et al. Neurologic testing with somatosensory evoked potentials in idiopathic scoliosis. Spine **17**, 277-9 (1992).
18. Goldberg, C.J., Dowling, F.E., Fogarty, E.E. & Moore, D.P. Adolescent idiopathic scoliosis and cerebral asymmetry. An examination of a nonspinal perceptual system. Spine **20**, 1685-91 (1995).
19. Burwell, R.G. et al. Etiologic theories of idiopathic scoliosis: neurodevelopmental concept of maturational delay of the CNS body schema («body-in-the-brain»). Stud Health Technol Inform **123**, 72-9 (2006).
20. Burwell, R.G. Aetiology of idiopathic scoliosis: current concepts. Pediatric Rehabilitation **6**, 137-170 (2003).

Selected Publications

1. Deng M, Hui SC, Yu FW, Lam TP, Qiu Y, Ng BK, **Cheng JC**, **Chu WC**. MRI-based Morphological Evidence of Spinal Cord Tethering Predicts Curve Progression in Adolescent Idiopathic Scoliosis. **Spine J 2015** 1;15(6):1391-401

2. Schlosser TP, van Stralen M, Brink RC, **Chu WC**, Lam TP, Vincken KL, Castelein RM, **Cheng JC**. Three-dimensional characterization of torsion and asymmetry of the intervertebral discs versus vertebral bodies in adolescent idiopathic scoliosis. ***Spine*** **2014**;39(19):E1159-66.

3. Kong Y, Shi L, Hui SC, Wang D, Deng M, **Chu WC, Cheng JC**. Variation in anisotropy and diffusivity along the medulla oblongata and the whole spinal cord in adolescent idiopathic scoliosis: a pilot study using diffusion tensor imaging. ***AJNR. American journal of neuroradiology*** **2014**;35(8):1621-7.

4. Shi L, Wang D, Hui SC, Tong MC, **Cheng JC**, **Chu WC**. Volumetric changes in cerebellar regions in adolescent idiopathic scoliosis compared with healthy controls. ***Spine J*** **2013**;13(12):1904-11.

5. Wang D, Shi L, **Chu WC**, Burwell RG, **Cheng JC**, Ahuja AT. Abnormal cerebral cortical thinning pattern in adolescent girls with idiopathic scoliosis. ***NeuroImage*** **2012**;59(2):935-42.

6. Wang D, Shi L, **Chu WC**, Paus T, **Cheng JC**, Heng PA. A comparison of morphometric techniques for studying the shape of the corpus callosum in adolescent idiopathic scoliosis. ***NeuroImage*** **2009**;45(3):738-48.

7. Shi L, Wang D, **Chu WC**, Burwell GR, Wong TT, Heng PA, **Cheng JC**. Automatic MRI segmentation and morphoanatomy analysis of the vestibular system in adolescent idiopathic scoliosis. ***NeuroImage*** **2011**;54 Suppl 1:S180-8

8. **Chu WCW**, Man GC, Lam WW, Yeung BH, Chau WW, Ng BK, Lam TP, Lee KM, **Cheng JC**. A detailed morphologic and functional magnetic resonance imaging study of the craniocervical junction in adolescent idiopathic scoliosis. 2007 **Spine**;32(15):1667-74

9. **Chu WC**, Man GC, Lam WW, Yeung BH, Chau WW, Ng BK, Lam TP, Lee KM, **Cheng JC**. Morphological and functional electrophysiological evidence of relative spinal cord tethering in adolescent idiopathic scoliosis. ***Spine*** **2008**;33(6):673-80.

10. **Chu WC**, Man GC, Lam WW, Yeung BH, Chau WW, Ng BK, Lam TP, Lee KM, **Cheng JC**. A detailed morphologic and functional magnetic resonance imaging study of the craniocervical junction in adolescent idiopathic scoliosis**.** ***Spine*** **2007**;32(15):1667-74.

Cerebral morphometric and functional abnormalities studied in adolescent idiopathic scoliosis. (MORFOSIA)

Jean-Edouard Loret
Christophe Destrieux

Two clinical and scientific units collaborate to run this study:
-The Pediatric Orthopedic Department of the Centre Hospitalier Régional Universitaire de Tours, which is nationally recognized for the management of Adolescent idiopathic scoliosis (AIS) patients.
- The Brain and Imaging research unit is a joint laboratory between INSERM and Université François Rabelais de Tours, which includes 5 research teams. The neuroanatomy-neuroimaging group, part of team 5, is skilled in the automated segmentation of the human cerebral cortex (1–3). In the frame of the FIBRATLAS project, selected during the Agence Nationale de la Recherche 2014 campaign, this group also aims at validating MRI tractography of the brain white matter. For this purpose, it uses 2 approaches based on test objects (4) and dissection (5). It includes 2 anatomists, one postdoc, two research engineers and 2 PhD students. This group collaborates with 2 leading laboratories in the field of MR imaging: the Martinos Center for Biomedical Imaging (Massachusetts General Hospital, Harvard Medical School, MIT, Charlestown, MA, USA), and Neurospin (CEA-Saclay, France).

Several complex spinal deformities are designed as AIS but may possibly derive from distinct pathophysiologic mechanisms. Various models were proposed for the emergence and development of AIS, none of them being fully convincing ; AIS probably results from variable interactions between genes and «environment», for instance hormonal or metabolic disorders, abnormal growth of the axial skeleton, or abnormalities of the central nervous system.
Several pathophysiological theories involving the central nervous system were indeed proposed : AIS indicate with disturbances of proprioceptive or sensory perception and/or with integration of this information.
This would result in an abnormal body mental representation, responsible for sensorimotor asymmetry that may promote or cause the deformation. Several imaging studies in AIS indeed plead for morpho-functional abnormalities of the structures involved in perception and sensory integration, as well as in control and motor coordination: corpus callosum (6,7), internal capsule (7), cerebral cortex (8,9). However, studies are rare and largely contradictory, possibly due to significant methodological limitations, particularly in terms of sample heterogeneity, several types of AIS usually being included

Hypothesis

We hypothesize that AIS is associated with subtle morpho-functional changes in cortical and white matter that may be studied by Magnetic Resonance Imaging. Such changes are clinically relevant since they may later be used as biomarkers for AIS diagnosis and classification.
Our principal aim is to study cortical and subcortical morphometric changes in the most frequent subtype of AIS (right thoracic). Our secondary objective is to prospectively study properties of the white mater and activations of sensory-motor networks in the same population as compared to control.
For this purpose, we will include 2 groups of 14 to 16 year-old right-handed female subjects, 8 with right thoracic AIS (Cobb angle between 20 and 40°), and 8 controls without scoliosis. This number of subjects is obviously not sufficient to get statistically significant results but was chosen to demonstrate the feasibility of this protocol, while limiting its cost.
These subjects will be scanned on a 3T Siemens Verio

MRI to get 3 types of information:
- A 3D-T1 weighted MRI will be processed using the FreeSurfer software. This software provides unfolded reconstruction of the cerebral cortex, maps of the cortical thickness, and an automated parcellation of the cortical surface in about 75 cortico-gyral structures (1). These tools will be used to compute individual statistics on cortical volume, and to compare both groups.
- A diffusion weighted MRI, which uses the diffusion properties of water molecules in the brain to study its microstructure. This technique is especially interesting to study white matter tracts of the brain connecting different cortical and subcortical structures. It will be used to reconstruct the main white matter tracts and to compute parameters linked to their properties (for instance Fraction of Anisotropy, Apparent Coefficient Diffusion…).

- Finally we will use functional MRI, which indirectly studies local changes in the brain vascularization, linked to functional activations. We will compute activation maps during a motor task of the hands. These data will then be used to compute asymmetry indices between both hemispheres, and to compare both groups of subjects. The functional connectivity in motor and sensory network will be studied using the evaluation of the signal covariance during the same task and at rest.

Results

Inclusion of patients and subjects are in progress and morphofonctional data is currently being processed. No statistical analysis is possible for now, given the partial inclusion of subjects. We nevertheless present preliminary individual results obtained in fMRI (Figure 1), in cortical segmentation (Figure 2), and tractography (Figure 3) for control subjects.

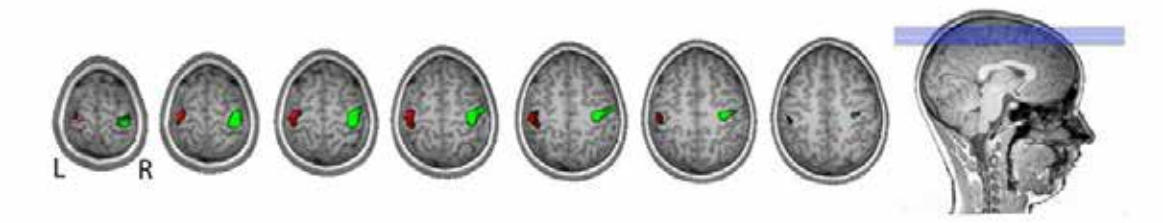


Figure 1: fMRI activation map during a motor task using hands versus rest in a normal subject
The green area, located in the right (R) precentral cortex, was activated during a motor task using the left hand. The red one, left precentral (L), was activated during the same task performed on the right side. Significance p <0.05 corrected (False Discovery Rate). Analysis General Linear Model (GLM) with SPM5

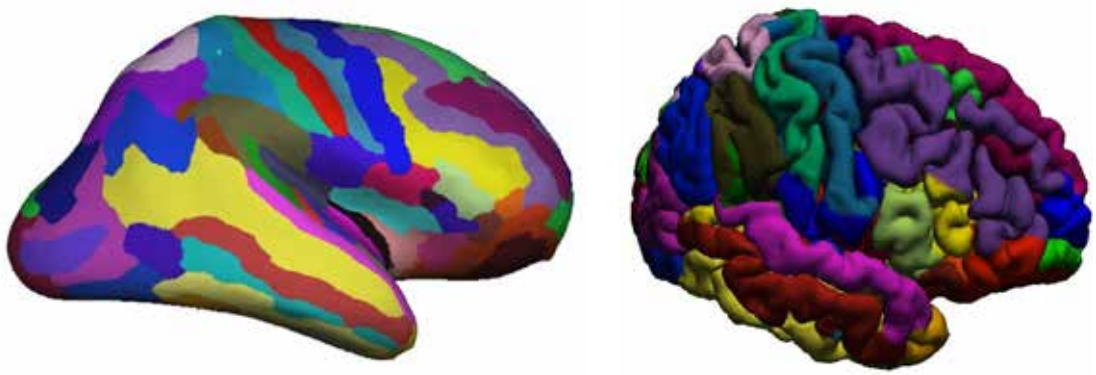


Figure 2: lateral views of the right hemisphere of a normal subject.
Both views are mathematical reconstruction of the same individual. Each color refers to one out of 75 sulco-gyral structures automatically segmented by the 5.3 freesurfer software using an atlas our group participates in developping (1). The right panel shows the «pial» representation of the cortex, whereas the left one displays the inflated representation of the gray-white interface

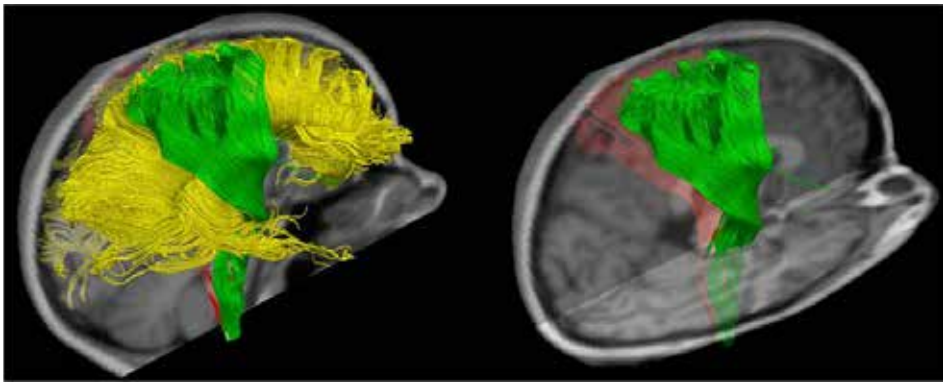


Figure 3 tractography of a normal subject
Each colored «fiber» shows a preferential pathway of water diffusion along white matter fibers. The right (green) and left (red) corticospinal tracks are shown on the right panel. The corpus callosum (yellow) was added on the left panel. Analysis was performed with the Trackvis software

References

1. Destrieux C, Fischl B, Dale A, Halgren E. Automatic parcellation of human cortical gyri and sulci using standard anatomical nomenclature. Neuroimage. 2010;53:115.

2. Fischl B, van der Kouwe A, Destrieux C, Halgren E, Segonne F, Salat DH, et al. Automatically parcellating the human cerebral cortex. Cereb Cortex. 2004;14(1):1122.

3. Landre L, Destrieux C, Baudry M, Barantin L, Cottier JP, Martineau J, et al. Preserved subcortical volumes and cortical thickness in women with sexual abuse-related PTSD. Psychiatry Res. 2010;183(3):1816.

4. Filipiak I, Destrieux C, Cottier J-P, Barantin L. Development of an anisotropic phantom for quality improvement of DTI and tractography algorithms in clinical practice. Poster présenté à: RSNA; 2013; Chicago.

5. Zemmoura I, Serres B, Andersson F, Barantin L, Tauber C, Filipiak I, et al. FIBRASCAN: A novel method for 3D white matter tract reconstruction in MR space from cadaveric dissection. NeuroImage. déc 2014;103:10618.

6. Liu T, Chu WC, Young G, Li K, Yeung BH, Guo L, et al. MR analysis of regional brain volume in adolescent idiopathic scoliosis: neurological manifestation of a systemic disease. J Magn Reson Imaging. 2008;27(4):7326.

7. Shi L, Wang D, Chu WCW, Burwell RG, Freeman BJC, Heng PA, et al. Volume-based morphometry of brain MR images in adolescent idiopathic scoliosis and healthy control subjects. AJNR Am J Neuroradiol. août 2009;30(7):13027.

8. Domenech J, García-Martí G, Martí-Bonmatí L, Barrios C, Tormos JM, Pascual-Leone A. Abnormal activation of the motor cortical network in idiopathic scoliosis demonstrated by functional MRI. Eur Spine J Off Publ Eur Spine Soc Eur Spinal Deform Soc Eur Sect Cerv Spine Res Soc. juill 2011;20(7):106978.

9. Wang D, Shi L, Chu WC, Burwell RG, Cheng JC, Ahuja AT. Abnormal cerebral cortical thinning pattern in adolescent girls with idiopathic scoliosis. Neuroimage. 2012;59(2):93542.

Neurological causes of Idiopathic Scoliosis

Dominique Rousié, PhD, neurosciences
Olivier Joly, PhD, neurosciences

The Ariane thread of our research has been the neurological causes of Idiopathic Scoliosis (IS). Our study focused on three different projects. - IS anatomical Head and Brain anomalies, -IS Anatomical and Physiological anomalies of the vestibular system, -IS corpus Callosum anomalies and consequences.
These three studies have a second goal : to determine the phenotype of Idiopathic Scoliosis.
Over the course of time, multiple collaborators have been included: Professor Alain Berthoz, Collège de France Paris, Professor Jean Delaire , maxillo-facial surgeon Nantes, Jean Claude Baudrillard, neuroradiologist C.H.Arras, Jean Paul Deroubaix, ENT Hôpital de Béthune, Paola Salvetti ophtalmologist Nordvision Lille , Jean Philippe Woillez, ophtalmologist CHU Lille, Edit Franko neurologist, University College London UK, Patrice Jissendi, MD 3T MRI researcher CHRU Lille, Maxime Rousié MD, Univ libre Bruxelles Belgique, Nancy Hadley Miller, MD University of Colorado Denver USA

Study 1 : Head and brain asymmetry in idiopathic scoliosis

This first part has been carried out by Dominique Rousié and Olivier Joly under the direction of Professor Alain Berthoz and Laboratoire de physiologie de la perception et de l'action, Collège de France Paris V, France.

Background and hypothesis

Several authors had underlined links between IS and brain asymmetry (1, 2, 3). Others between IS and functional Right/Left asymmetries (4, 5, 6). The development of the brain induces shape and volume of the skull. In case of brain asymmetry, the different bony part of the skull (face, calveria, basicranium) are simultaneously affected. Our goal was to measure IS shape and spatial orientation of the Basicranium compared to non scoliotic controls keeping in mind that craniofacial asymmetry(CFA) involves vestibular organs and orbit spatial asymmetries with physiological consequences on the oculolabyrinthic system and postural outputs.

Methods

To measure CFA asymmetry we used an original internal referential (fig1) implemented in Anatomist / Brainvisa (http://brainvisa.info/). MRI acquisition: Excite MRI from General Electric with 1.5T. magnet and head coil. A T2-weight sequence was used in 3D mode acquisition. A Fiesta fast imaging employing steady state sequence with following parameters: orientation: axial , FOV=250.250mm, matrix dimension MD=256x256, repetition delay TR=5ms, flip angle= 65°, number of echoes NE=3, swip with <<<sw=31;25kHz, slice thickness SIT= 1mm, number of slice NSI= 192, zero Interpolation processing 512 ZIP. Brainvisa processing: 1. Import and convert dicom images to "GIS Brainvisa format". 2. Select and save chosen points used to referential construction. For each selected point, Brainvisa gives 3D coordonates referred to MRI volume, 3. 2 marker points P &P' are selected on right and left of the posterior basicranium (junction between the auditory meatus and of semi-circular canals), 4. To define the 2 planes of the referential we calculate the plane equation from Cartesian coordinates of 3 points A (xa ya za), B (xb yb zb), C (xc yc zc).
Cancelling the matrix determinant we obtain

$$M = \begin{bmatrix} x - x_a & y - y_a & z - z_a \\ x_b - x_a & y_b - y_a & z_b - z_a \\ x_c - x_a & y_c - y_a & z_c - z_a \end{bmatrix}$$ ax+bx+cz+d=0

The programme calculates distance from these markers to sagital, axial and frontal planes: our measurement is the difference of distance between P and P' to the planes.

Results

The test-retests correlation(Rho) was calculated to examine the reproducibility of measurements.

This analysis showed significant correlation of the method (intra-class coef. = 0.8, $P<0.05$).

Student tests completed with the Satterthwaite test validated the results (SG= scoliotic group, CG= control group)

groups	Directions	Mean	Median	Std Dev	Minimum	Maximum
SG(n=93)	Lateral	3.6459239	3.3250000	2.4309742	0.100000	11.8770000
	A.P.	*4.1144674	3.7420000	2.6955483	0.059000	16.6110000
	Vertical	**1.7692826	1.4010000	1.4487562	0	7.8460000
CG(n=32)	Lateral	1.8808437	1.8035000	1.6359105		6.5680000
	A.P.	*2.0809375	1.8450000	1.8145113		7.4570000
	Vertical	**1.2729063	1.1725000	0.9330775		3.0060000

These measurements show that the basicranium is never symmetrical in humans. The modelisation revealed a torque movement of the base previously described by Le May as petalia frontalis movement. This movement, chiefly involves the posterior part of the basicranium and reflects asymmetry of the cerebellum (fig1): IS patients have a significant increase of this movement .

This anomaly has been previously described but not measured by Burke (7) Lundstrom(8) and Previc(9). The torque movement causes an anteroposterior deformation of the skull and face: this anomaly is always visible during clinical examination of the patient by comparing right and left horizontal level of ears and orbits. A correlation between severity of scoliosis and amplitude of this asymmetry would be done.

This finding also precise the phenotype of IS: we demonstrated that IS have a specific craniofacial asymmetry involving vestibular organs and orbits spatial asymmetries with physiological consequences on the oculolabyrinthic system and postural outputs.

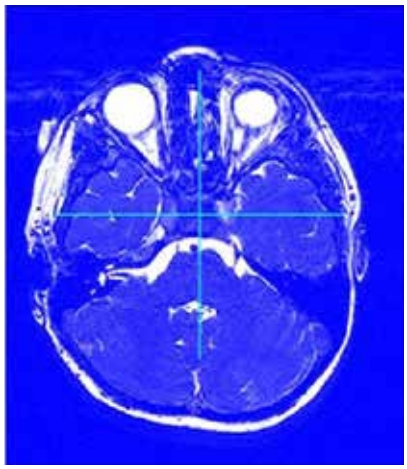


Fig 1 : IS posterior basicranium assymetry reflecting cerebellum assymetry.

References

1. Geissele AE: Magnetic resonance imaging of the brain and stem in adolescent idiopathic scoliosis, Spine 1991, Jul; 16(7):761-3.

2. Kertesz A,Polk M: Anatomical asymmetries and functional laterality, Brain 115: 589-605

3. Delaire J: Syndromes malformatifs craniofaciaix in Traité de pathologies buccales et maxillofaciales, De Boeck University, Bruxelles, ed.91, 2.

4. Yamada K, Yamamoto H, Nakagawa Y, Tezuka S, Kawata S:Etiology of idiopathic scoliosis, Clin Orthop. 1984 Apr ;(184):50-7.

5. Burwell RG, Dangefield PH: Etiologic theories of idiopathathic scoliosis: neurodevelopmental concepts to be evaluated, Stud Health Inform, 2002; 91:15-9.

6.Shimode M, Ryouji A, Kozo N: Asymmetry of premotor time in the back muscles of AIS, Spine 2003 Nov 15; 18(22):2535-9.

7. Burke PH: Stereophotogrammetric measurement of normal facial asymmetry in children, 1971, Human Biology; 43: 536-548.

8. Lundstrom A:Some asymmetries of the dental arches, jaws and skull and their etiological significance. American J. of orthodontics, 1961, 47:81-106.

9. Previc FH: A general theory concerning the prenatal origins of cerebral lateralization in Humans,1991, Psychological Review,98,3: 299-334.

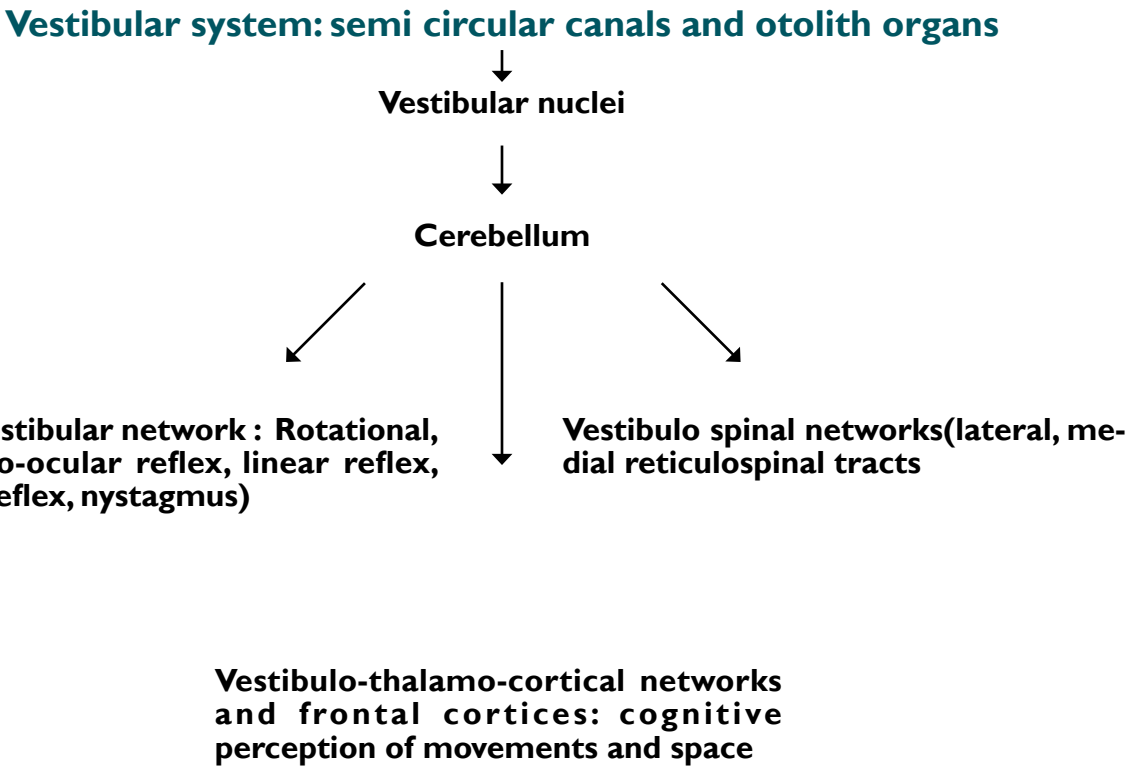
Study 2 : Idiopathic scoliosis and oculo-labyrinthic System

This second part was carried out by Dominique Rousié and Olivier Joly under the direction of Professor Alain Berthoz, Laboratoire de physiologie de la perception et de l'action, Collège de France Paris V, France with the collaboration of Dr. Baudrillard, Dr. Deroubaix, Dr. Salvetti, Dr. Woillez.

Background and hypothesis

Posture, movements and equilibrium of the skeleton depend on brain outputs sent to muscles. Many studies have demonstrated, in IS, a lack of equilibrium and difficulties in sustaining posture (1, 2, 3).

We focused on the oculo-labyrinthic system because vestibulo-spinal outputs acting on tonic muscles (spine muscles are chiefly tonic muscles).



Oculolabyrinthic system investigations

-Eye investigations: We focused on a specific eye anomaly rarely studied by ophthalmologists and spine clinicians: ocular torsion (OT) which is a rotation of the eye axis in the frontal plane, never seen in clinical examinations because the iris is symmetric and round on both eyes. OT is a mirror of neural dysfunction of the cerebellum and/or vestibular nuclei and/or vestibular organs (5, 6, 7, and 8)

-Vestibular function investigation: we focused on canals and otolithic function using specific tests (4, 9, and 10)

Methods

1. Eyes investigation: O.T can be measured thanks a retinograph widely used by ophthalmologists in detection of retina anomalies but in case of OT the head must be positioned right in gravitational axis (to avoid the reflex of counterclockwise rotation). In a previous study, we evaluated the normal foveal position measured in degree in frontal plane on 100 normal subjects (14.). A foveal position higher than 3° referred to a horizontal line is abnormal and corresponds to OT. A difference of 3° or more between right and left position is considered as significative dysfunction of the oculo vestibular system.

2. We developed a patented MRI 3D reconstruction program for visualizing membranous part of vestibular organs. This program, working with native MRI data, does not use included algorithms of reconstruction (as ZIPs) of MRI machines: possible source of false images.

The knowledge of the internal structure of vestibular organs is essential: the signal received by the hair cells, transmitted to the brain determines the vestibulo- outputs to eyes and spine.

The main originality of this program is the increase of size images of membranous canals allowing discovery of anomalies as intra-membranous canal stenosis, dysplasia and abnormal inter-canals connections (4).

-Protocol for vestibular examination: test of spontaneous nystagmus, rotator y impulse test, rotatory velocity steps, saccades and pursuits tests.

Results and discussion

1. We discovered in IS patients high percent malformations of the lateral semi-circular canals compared to controls (70%) (12)(fig.1) in a significant number of cases, lateral canal was abnormally connected with posterior canal (LPCC): In a cohort of 445 non selected subjects we found 67/445(15%) of LPCC. In 95 IS patients we found 52/95(55%) of LPCC. By testing the vestibulo- ocular reflex (VOR), in horizontal and vertical planes in case of LPCC, during a counterclockwise horizontal or post clockwise horizontal rotation, added to expected horizontal nystagmus, we found an unexpected upbeat nystagmus induced by the ampullofugal displacement of the fluid in the posterior canal, giving the proof that, in IS vestibular anatomical anomalies can be implicated in postural and balance dysfunction.

Because of the development timing, these lateral canal anomalies are associated to utricle malformations (14) highly implicated in tonic outputs to spine muscles. These results are in accord with Lambert study (13). A targeted questionnaire to IS children highlighted vestibular symptoms: -delay for walking,-delay for riding bicycle, - frequent transport sickness, instability but no rotatory vertigo , spatial disorientation in new environment,- IS patients always have a head tilt on the side of the vestibular anomaly.

2. Eyes investigation: We discovered that IS are always affected with significant OT compared to controls but no correlation between severity and type of scoliosis has been found (15).

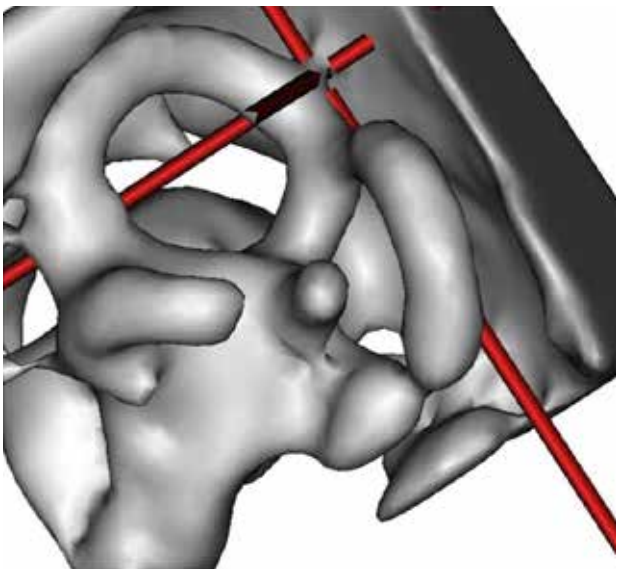


Fig 1: malformation of the left lateral (horizontal) semicircular canal in an IS patient

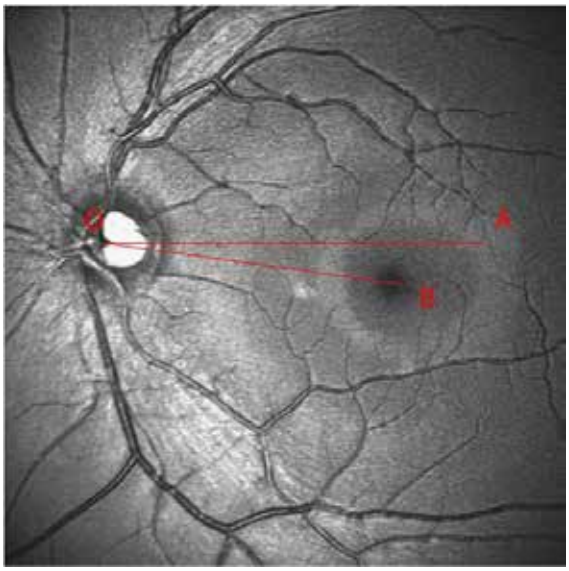


Fig2: a= horizontal line of reference; o= centre of macula; b=foveola. AOB angle measured the degree of rotation of the eye. AOB is measured on each eye

1. Bacsi AM, Colebatch JG: Evidence for reflex and perceptual vestibular contribution to postural control, Exp Brain Res2004, Aug 18.
2. Berthoz A, Rousié D: Physiopathology of otolith-dependent vertigo, contribution of the cerebral cortex and consequences in craniofacial asymmetry DV IN ORL 58, 48-67 2000.
3. Yamamoto H, Yamada K: Equilibral approach to scoliosis posture Agressologie 1976, 17: 61-66.
4. Rousié D.L., Baudrillard J Cl, Joly O, Deroubaix JP, Berthoz A. : Abnormal connection between lateral and posterior semi-circular canals revealed by a new modeling process. Origin and physiological consequences, in Basic and clinical aspects of vertigo and dizziness, Ann. N.Y. Acad. Sci. 1164:455-457; 2009;
5. Chin S: ocular torsion produced by unilateral lesion of the cerebellar flocculus in alert cat, Curr eye res, 2002 Sept: 25(3): 133-8.
6. Brandt t: Vestibular syndroms in the roll plane, topographic diagnosis from brainstem to cortex; ann. Neurol. 1994; 36:337-47.
7. Leigh & Zee: the vestibular optokinetic system, Neurology of eyes movements 1999, 2:19-89.
8. Leigh & Zee: Skew deviation and ocular tilt reaction (OTR). Diagnosis of central disorders of ocular motility in Neurology of eyes movements contemporary Neurology Series, 10; 1:4-18.
9. Hamalgy G.: Control of head-eye posture in the roll plane, in the head Neck Sensory motor sytem Berthoz A, Graf W Vidal P eds Oxford Univ Press 1992: 635-637.
10. Corthoys IS: Eye movements produced by utricular & saccular stimulation, Aviat.Environ. Med. Supl.88: A 192-7.
11. Wiener-Vacher SR, Mazda (1998): Assymetric otolith vestibule-ocular responses in children with idiopathic scoliosis J.Ped.: 132 1028-1032.
12. Rousié D., Joly O: Semi-circular canals anomalies and scoliosis Arxiv 2009: userID:1003.0836, password: xjwdi
13. Lambert FM, Malinvaud D et al: Vestibular asymmetry as cause of idiopathic scoliosis. A possible answer from Xenopus J Neurosci. 2583-09; 2009.
14. Mazan S, JaillardD,Baratte Bl, Janvier Ph: Otx1 gene-controlled morphogenesis of the horizontal semi-circular canal and origin of characteristics of gnathostome , Evolution and development 2000, 4 186-193.
15. Woillez J Ph: review of 100 cases of foveal position in frontal plane, North American neuro-ophtalmological Society 2012 San Antonio TX.

Study 3 : A new approach to corpus callosum anomalies in idiopathic scoliosis using diffusion tensor magnetic resonance imaging

This third part was carried out by Olivier Joly, Dominique Rousié, Edit Franko, Patrice Jissendi, Maxime Rousié

In the last decade, human magnetic resonance imaging (MRI) morphometry studies (e.g. cortical, thickness, 2D shape of the corpus callosum) have aimed to investigate the potential contribution of the central nervous system in the etiopathogenesis of IS. Recent developments in diffusion tensor imaging (DTI) allow us to extend the previous work to the study of white matter microstructure. Here, we hypothesized that part of the corpus callosum could show a difference in white matter microstructure in IS patients as compared to healthy controls. Diffusion tensor imaging (DTI) can be used to determine the orientation of cerebral white matter fibres [1] and to derive the fractional anisotropy (FA) within each voxel of the image [2]. FA is calculated from the eigenvalues of the diffusion tensor. It is a scalar value between zero and one that describes the degree of directionality of water diffusion through tissue.

Methods

Ten girls with IS were recruited (10–24 years of age, median age 14, right-handed) from the CHRU Hospital of Lille. The scoliosis and its progression were assessed with a repeated measurement at 6 months of the degree of angulation measured with EOS 3D reconstructions [3]. The deformity was considered progressive if it increased by at least three degrees at the thoracic level. Inclusion Criteria for the patient group were Cobb angle e»15°. All the patients in this study belong to the two most common types: right thoracic and right thoracic-left lumbar. Data from 49 (10–18 years of age,median age 14) right-handed healthy girls as control subjects were obtained from the NIH Pediatric MRI Data Repository created by the NIH MRI Study of Normal Brain Development.

Magnetic resonance images were acquired at 3Tesla (Achieva, Philips Medical Systems) using parallelimaging SENSE-Head-8 channels coil. The sequences included a T1-weighted 3D acquisition,with echo time (TE) = 3.301 ms, flip angle = 98, repetition time (TR) = 7.199 ms, slice thickness = 1 mm,matrix = 256 9 256 and a DTI spin-echo echoplanar image (SE-EPI) 78 axial slices, slice thickness = 2 mm, TR = 13,000 ms, flip angle = 908, TE= 55 ms,matrix = 128 9 128 and along 32 isotropically distributed directions with b values of 1,000 s/mm2. The DTI acquisition was performed with isotropic resolution of 2 mm.The MR acquisitions in patients lasted for about 30 min (10 and 20 min for the T1 and the diffusion MR sequence, respectively. We used the ICBM-DTI-81 (International Consortium ofBrain Mapping) atlas as as template (http://www.loni.ucla.edu/Atlases/). The FA maps in patients were compared with those in controls using Mann–Whitney test.

Results

Using a voxel-based two-sample t test analysis implementedin SPM, we looked for significantly lower FAs in IS patients as compared to controls (Fig. 1). The map revealed significant voxels in the anterior part of the midbody of the corpus callosum which corresponds mainly to the region II from the modified Witelson scheme [4]. This region corresponds to premotor cortex close to accessory motor cortex (Fig2) These results are in accord with Domenech (5)

Discussion

We found that the body had significantly lower FA in patients with IS than in controls. In particular, the anterior part of the body (region II) showing a significant difference in both the voxel and ROI-based analyses. Brain development is characterized by both progressive myelination and regressive pruning processes.

These two factors can modulate substantially the FA values. Myelination increases the outer diameter of the axon and decreases its permeability, therefore, increasing the FA. In contrast, elimination of axons during development (pruning) decreases the packing density and the number of axons in a voxel, therefore, decreasing FA. These two processes are particularly active during childhood and adolescence. They depend on genetic and environmental factors. The co-occurring myelination and pruning with other changes in the white matter microstructure during childhood was found to result in a net increase of FA in many brain regions [6].

One of these regions is the corpus callosum, shows early and rapid change of FA with age. Lebel and colleagues [6] found that the genu and splenium of CC reached 90 % of their maximum FA value by 11 years of age.

This suggests that in most of our patients, the main part of myelination and pruning in the CC has already finished by the onset of the scoliosis. The found difference between the patient and the control groups, therefore, might indicate that the alteration of white matter microstructure reflected by the FA precedes the spine deformity. Although, both myelination and pruning occur in the adult brain as well [7,8] this has only a small effect on the FA value [6], which further supports that the callosal change is not a consequence of the disease.

These results already indicate the possible involvement of abnormal brain development in the aetiopathogenesis of idiopathic scoliosis; however, further research involving more patients and different types of scoliosis is needed to draw clear conclusions.

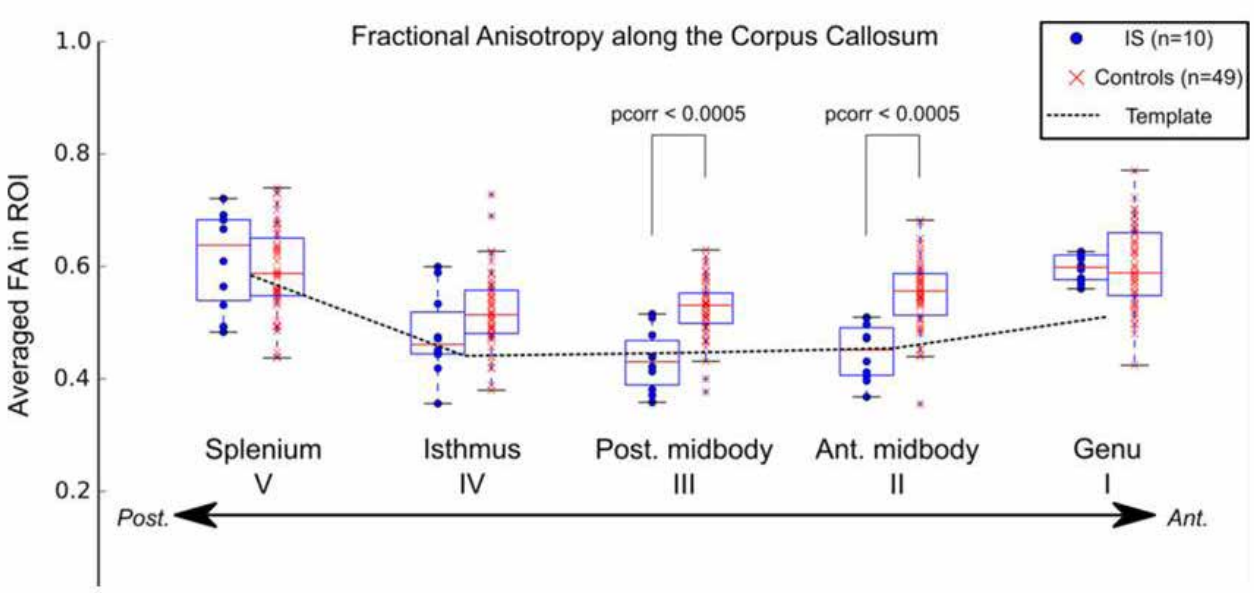


Fig. 1 Distribution of Fractional Anisotropy along the corpus callosum in the 10 IS patients (blue circle for individual subjects) and 49controls (red crosses for individual subjects). Box plots show the lower to upper quartile values and the median. The black dotted line represents the FA values of the ICBM-DTI-81adult template.

References

1.Wakana S, Jiang H, Nagae-Poetscher LM et al (2004) Fiber tract basedatlas of human white matter anatomy. Radiology 230:77–87. doi:10.1148/radiol.2301021640

2. Basser PJ, Pierpaoli C (1998) A simplified method to measure the diffusion tensor from seven MR images. Magn Reson Med 39:928–934

3. Rousseau M-A, Laporte S, Chavary-Bernier E et al (2007) Reproducibility of measuring the shape and three-dimensional position of cervical vertebrae in upright position using the EOS stereoradiography system. Spine (Phila Pa 1976) 32:2569–2572. doi:10.1097/BRS.0b013e318158cba2 4. Hofer S, Frahm J (2006) Topography of the human corpus callosum revisited–comprehensive fiber tractography using diffusion tensor magnetic resonance imaging. Neuroimage 32:989–994. doi:10.1016/j.neuroimage.2006.05.044

5. Domenech J, Garcia-Marti G, Marti-Bonmati L et al (2011) : Abnormal activation of the motor cortical network in idiopathic scoliosis demonstrated by functional MRI. Eur Spine J20:1069–1078. doi:10.1007/s00586-011-1776-8

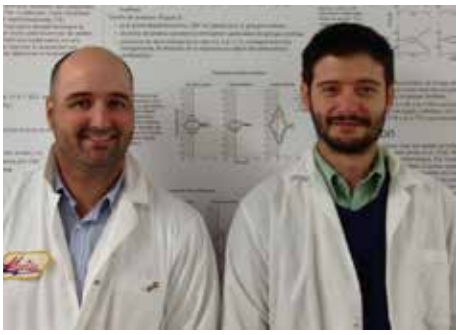
6. Lebel C, Walker L, Leemans A et al (2008) Microstructural maturation of the human brain from childhood to adulthood. Neuroimage 40:1044–1055. doi:10.1016/j.neuroimage.2007.12.053

7. Yamahachi H, Marik SA, McManus JNJ et al (2009) Rapid axonal sprouting and pruning accompany functional reorganization in primary visual cortex. Neuron 64:719–729. doi:10.1016/j.neuron.2009.11.026

8. Ishibashi T, Dakin KA, Stevens B et al (2006) Astrocytes promote myelination in response to electrical impulses. Neuron 49:823–832doi:10.1016/j.neuron.2006.02.006

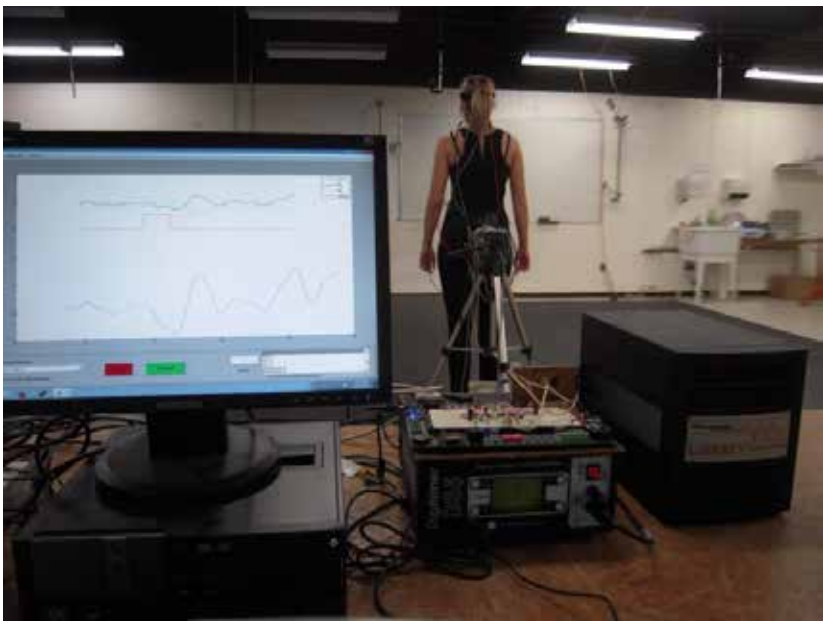
Sensorimotor transformation is altered in adolescent idiopathic scoliosis patients

Professor Martin Simoneau obtained his PhD in neuromechanics from Laval University in 2000 and thereafter he was a postdoctoral fellow at Northwestern University for two years. He joined the Faculty of medicine at Laval University following his postdoctoral training. His research group takes a multidisciplinary approach combining experimental approaches with sophisticated signals analysis and modelling. His laboratory (Laboratory of neuromechanics and motor control) focuses on sensorimotor processes that control human movement and balance and their disorders resulting from damage to the central nervous system.



Professor Martin Simoneau and DoctorJean-Philippe Pialasse

Our main lines of investigation are aimed at understanding the mechanisms that control and integrate whole-body actions such as standing, walking and reaching. We are mainly interested in how the neural processes combine sensory information from vestibular organs, eyes, muscles and skin to select motor commands and control movement. We used a wide range of neurophysiological and psychophysical techniques to investigate fundamental human physiology and pathophysiology relevant to study adolescent idiopathic scoliosis. Furthermore, we use computational modelling to investigate the complex multi-component neural system involved in motor control (Pialasse et al., 2015). Most of our work is basic research as we are trying to understand how the brain controls movement in healthy individuals and in adolescent idiopathic scoliosis patients. This is a worthwhile challenge. We have several collaborations with clinicians and researchers in Canada, United States and Europe.



Experimental set-up allowing to assess the vestibulomotor control of AIS patients (Pialasse et al., 2015)

References related to the assessment of the sensorimotor integration in adolescent idiopathic scoliosis patients (**bold items funded by Fondation Yves Cotrel – Institut de France**, *italicized items indirectly benefit as the study used research equipment funded by Fondation Yves Cotrel – Institut de France*)

Beaulieu, M., Allard, P., Simoneau, M., Dalleau, G., Hazime, F. A., & Rivard, C. H. (2010). Relationship between oscillations about the vertical axis and center of pressure displacements in single and double leg upright stance. *American Journal of Physical Medicine and Rehabilitation, 89*(10), 809-816.

Beaulieu, M., Toulotte, C., Gatto, L., Rivard, C. H., Teasdale, N., Simoneau, M., & Allard, P. (2009). Postural imbalance in non-treated adolescent idiopathic scoliosis at different periods of progression. *European spine journal, 18*(1), 38-44.

Blouin, J., Guillaud, E., Bresciani, J. P., Guerraz, M., & Simoneau, M. (2010). Insights into the control of arm movement during body motion as revealed by EMG analyses. Brain Research, 1309, 40-52.

Mackrous, I., & Simoneau, M. (2011). Visuo-vestibular interaction: predicting the position of a visual target during passive body rotation. Neuroscience, 195, 45-53.

Mackrous, I., & Simoneau, M. (2014). Generalization of vestibular learning to earth-fixed targets is possible but limited when the polarity of afferent vestibular information is changed. Neuroscience, 260, 12-22.

Mackrous, I., & Simoneau, M. (2015). Improving spatial updating accuracy in absence of external feedback. Neuroscience, 300, 155-162.

Pialasse, J. P., Descarreaux, M., Mercier, P., Blouin, J., & Simoneau, M. (2015) Adolescents with idiopathic scoliosis rely more than healthy adolescents on vestibular information to control their balance. *Plos ONE, 10, e0143124*.

Pialasse, J. P., Mercier, P., Descarreaux, M., & Simoneau, M. (2015a). Assessment of sensorimotor control in adults with surgical correction for idiopathic scoliosis. *European Spine Journal, in review*.

Pialasse, J. P., Mercier, P., Descarreaux, M., & Simoneau, M. (2015b). Assessment of sensory reweighting in young adults with idiopathic scoliosis. *Journal of manipulation physiological therapeutics, in review*.

Pialasse, J. P., Descarreaux, M., Mercier, P., & Simoneau, M. (2015). Sensory reweighting is altered in adolescent patients with scoliosis: evidence from a neuromechanical model. *Gait and Posture, 42, 558-563*.

Pialasse, J.-P., Descarreaux, M., Mercier, P., Blouin, J., & Simoneau, M. (2012). Sensorimotor integration in adolescent idiopathic scoliosis patients *Recent advances in scoliosis*: InTech.

Pialasse, J. P., Laurendeau, S., Descarreaux, M., Blouin, J., & Simoneau, M. (2013). Is abnormal vestibulomotor responses related to idiopathic scoliosis onset or severity?

During the last ten years our research team has been investigating the sensorimotor transformation of adolescent idiopathic scoliosis patients (AIS) and the ability of AIS patients to process vestibular information.This paper presents of summary of some of our studies supported by the Cotrel Foundation.

More than 3 decades ago, Sahlstrand et al. (1978) explored the effect of removed vision and/or attenuated plantar sole mechanoreceptor information (i.e., participants stood on a compliant support base) on balance control in AIS patients. Their results revealed that attenuating sensory information originating from mechanoreceptors of the soles and/or occluding visual information increased the sway area of AIS patients to a greater extent than that of the controls. One limitation of this study is that standing on an unstable support base (i.e., compliant surface) may exacerbate AIS patients' balance control problem.

Sensory reweighting and balance control in AIS patients

Studies in my laboratory expanded on the work of Sahlstrand et al. (1978) by deploying a different technique to manipulate lower limb proprioception: we applied tendon vibration to mask ankle proprioception (Simoneau *et al.*, 2006). Furthermore, we assessed the underlying cause of greater body

sway oscillations in AIS by quantifying the sway density curve during sensory deprivation (Baratto *et al.*, 2002). By controlling the availability of sensory information and quantifying the outcome, that is, the range and variability of the centre of pressure (CP) displacement, we could objectively ascertain the role of visual information and/or ankle proprioception on balance control. Overall, the results revealed that the range and variability of the CP of AIS patients were larger than those of the controls when ankle proprioception was attenuated. Furthermore, sway density curve analysis demonstrated that these observations could be explained by amplitude rather than balance control command variation.

These results were seen whether vision was available or not. It suggests that despite the availability of vision, AIS patients rely much more on ankle proprioception to scale the amplitude of their balance control commands than control participants. It could be hypothesized that AIS patients, compared to control participants, had difficulty in reweighting the gains of the vestibular system and the remaining sensory inputs after ankle proprioception alteration whether vision is available or not.

A key finding of human balance control is that the integration of sensory information appears to be dynamically regulated to adapt to changing environmental conditions and the availability of sensory information; this process is called sensory reweighting. In another study conducted in our laboratory, we assessed the ability of AIS patients to perform sensory reweighting (Simoneau *et al.*, 2006).

The framework to determine the ability of the brain to reweigh sensory information consists of monitoring balance stability during transient sensory perturbation, for example, when vision is removed and then becomes available again. In this circumstance, a decrease in balance stability (e.g., a sudden increase in body sway) could result from difficulties in dynamically reweighting sensory information when it is made available after a period of deprivation. In absence of sensory manipulation, AIS patients showed greater balance control impairment. Sway density curve analysis revealed that AIS patients' balance control commands were greater and more variable than those of the controls for mean distance and mean peak.

Furthermore, balance stability analysis after the reintegration of vision showed that AIS patients had greater CP RMS velocity than the controls; the balance control commands of AIS patients were much more variable than those of the controls. Balance stability analysis after reintegration of ankle proprioception when vision was available or not revealed that balance motor commands in AIS patients were much more variable than in the controls. Overall, these results suggest that AIS patients had difficulty to transform sensory orientation cues into corrective balance control commands.

Cortical vestibular integration and sensory reweighting

Although various studies have proven that some AIS patients have abnormal VOR (Manzoni & Miele, 2002), little attention has been devoted to assessing the capacity of AIS patients to integrate vestibular information for cognitive processing of space perception. Consequently, we investigated the ability of AIS patients to process vestibular information for space updating (Simoneau *et al.*, 2009). In this experiment, seated AIS patients and controls experienced torso rotations of different directions and amplitudes in the dark and produced saccades that would reproduce their perceived spatial characteristic of the rotations (vestibular condition). Furthermore, we controlled for possible alteration of the oculomotor and vestibular systems by measuring subject accuracy in performing saccades toward memorized peripheral targets in the absence of body rotation and gain of their vestibulo-ocular reflex. Overall, this study revealed that, compared to the controls, AIS patients underestimated the amplitude of rotations, indicating impairment of their ability to memorize and process vestibular signals. It is possible that severe spinal deformity was partly due to impaired vestibular information travelling from the cerebellum to the vestibular cortical network or alteration in the cortical mechanisms processing vestibular signals.

As a follow up of these previous studies, we assessed if sensory reweighting impairment was specific to somatosensory and visual systems or also involved the vestibular system (Pialasse et al., 2015). To do so, we adopted galvanic vestibular stimulation (GVS) to manipulate vestibular sensory information (i.e., semicircular canals and otoliths) and to induce a vestibular-evoked postural response. Binaural bipolar GVS stimulation, with

the head straight ahead, provokes body sway along the frontal plane towards the anode side (Day *et al.*, 1997). Results revealed that adolescent idiopathic scoliosis patients demonstrated greater lateral displacement and net lateral forces than controls during and immediately after vestibular stimulation. The analysis of the amplitude of the net lateral forces before body movement revealed that these patients' vestibulomotor response was similar to that of controls. This latest result suggests that the vestibulomotor response would not be involved in scoliosis onset.

Nonetheless, the larger body sway during and following vestibular stimulation confirms that AIS patients have difficulty reweighting sensory information.
Using a neuromechanical feedback control model, we could confirm that, compared to controls, adolescent idiopathic scoliosis patients assigned a larger weight to vestibular feedback information although the vestibular information was unreliable (Pialasse et al., 2015).

Overall, the present data extend previous findings by demonstrating that, both during and after vestibular stimulation, the balance control of AIS patients is altered compared to controls. Consequently, scoliosis onset could be related to abnormal sensory reweighting leading to altered sensorimotor transformation.

References

Baratto L, Morasso PG, Re C, Spada G. A new look at posturographic analysis in the clinical context: sway-density versus other parameterization techniques. Motor Control. 2002;6(3):246-70.
Day BL, Severac Cauquil A, Bartolomei L, Pastor MA, Lyon IN. Human body-segment tilts induced by galvanic stimulation: a vestibularly driven balance protection mechanism. J Physiol (Lond). 1997;500 (Pt 3):661-72.
Sahlstrand T, Ortengren R, Nachemson A. Postural equilibrium in adolescent idiopathic scoliosis. Acta Orthop Scand. 1978;49(4):354-65.
Simoneau M, Lamothe V, Hutin E, Mercier P, Teasdale N, Blouin J. Evidence for cognitive vestibular integration impairment in idiopathic scoliosis patients. BMC Neurosci. 2009;10:102.
Simoneau M, Mercier P, Blouin J, Allard P, Teasdale N. Altered sensory-weighting mechanisms is observed in adolescents with idiopathic scoliosis. BMC Neurosci. 2006;7:68.
Simoneau M, Richer N, Mercier P, Allard P, Teasdale N. Sensory deprivation and balance control in idiopathic scoliosis adolescent. Exp Brain Res. 2006;170(4):576-82.
Pialasse JP, Descarreaux M, Mercier P, Blouin J, Simoneau M. The Vestibular-Evoked Postural Response of Adolescents with Idiopathic Scoliosis Is Altered. PLoS One. 2015;10(11):e0143124.
Pialasse JP, Descarreaux M, Mercier P, Simoneau M. Sensory reweighting is altered in adolescent patients with scoliosis: Evidence from a neuromechanical model. Gait Posture. 2015;42(4):558-63.

Vestibular asymmetry as the cause of idiopathic scoliosis: A possible answer from xenopus

Pierre-Paul Vidal obtained his doctor's degree in 1978 and was awarded the Silver Medal of the State Medical Doctorate of that year.
In 1986 he obtained his PhD in Sciences.
He created :
- The Laboratory of Neurobiology of sensorimotor networks (UMR 7060) in 1997
- The Centre for Study of sensorimotor (CESEM UMR 8194) in 2010
- The Cognition and Action Group (UMR G **Cognac** Defense) in 2014

Parallel to his studies and his research, Pierre-Paul Vidal dedicated to teaching from 1976 to 2004.

Presentation of the research unit (Paris, France)
Pierre-Paul Vidal
Francois M. Lambert
David Malinvaud
Hans Straka

"COGNAC G" is a cooperative project that will investigate the long-term follow up of human groups (ethomics), which have in common to be engaged in complex behavioral tasks during a long stretch of time. These populations need to be followed in order to evaluate their training and once trained to check that their skills are operational. They also need to be to monitored carefully to avoid excessive pressures, which could lead to pathologies such as the burnout syndrome, overtraining, and PTSD. We propose to name these groups "High maintenance cohorts" or HMC. They are very diverse and, given the evolution of society, their number will inevitably rise. HMC includes military groups in active duty, athletes at high levels of competition, patients with neurological diseases, patients in reeducation, psychiatric patients, people with heavy chronic handicaps, very senior citizens, etc.

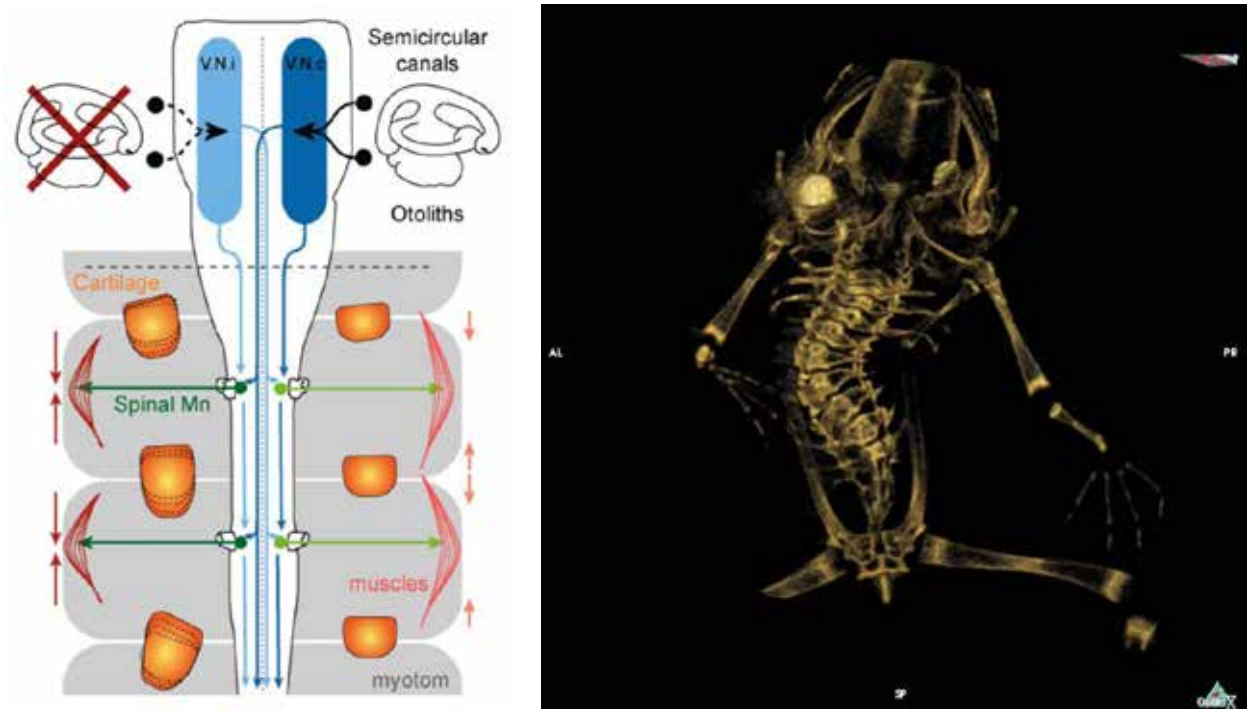
The COGNAC G project has three objectives:
• Our first objective is to learn how to monitor and quantify the behavior of the HMC defined above, both at the sensorimotor and cognitive level. These measurements to be efficient should be performed in real time, at correct sample frequencies, and above all should be non-invasive or at least minimally invasive. That is, the subjects under scrutiny should continue their ongoing activities with no problem.

• Our second objective is to investigate how to use these measurements to build and exploit large data banks on human behavior, which is necessary to do effective longitudinal follow-ups of any HMC.

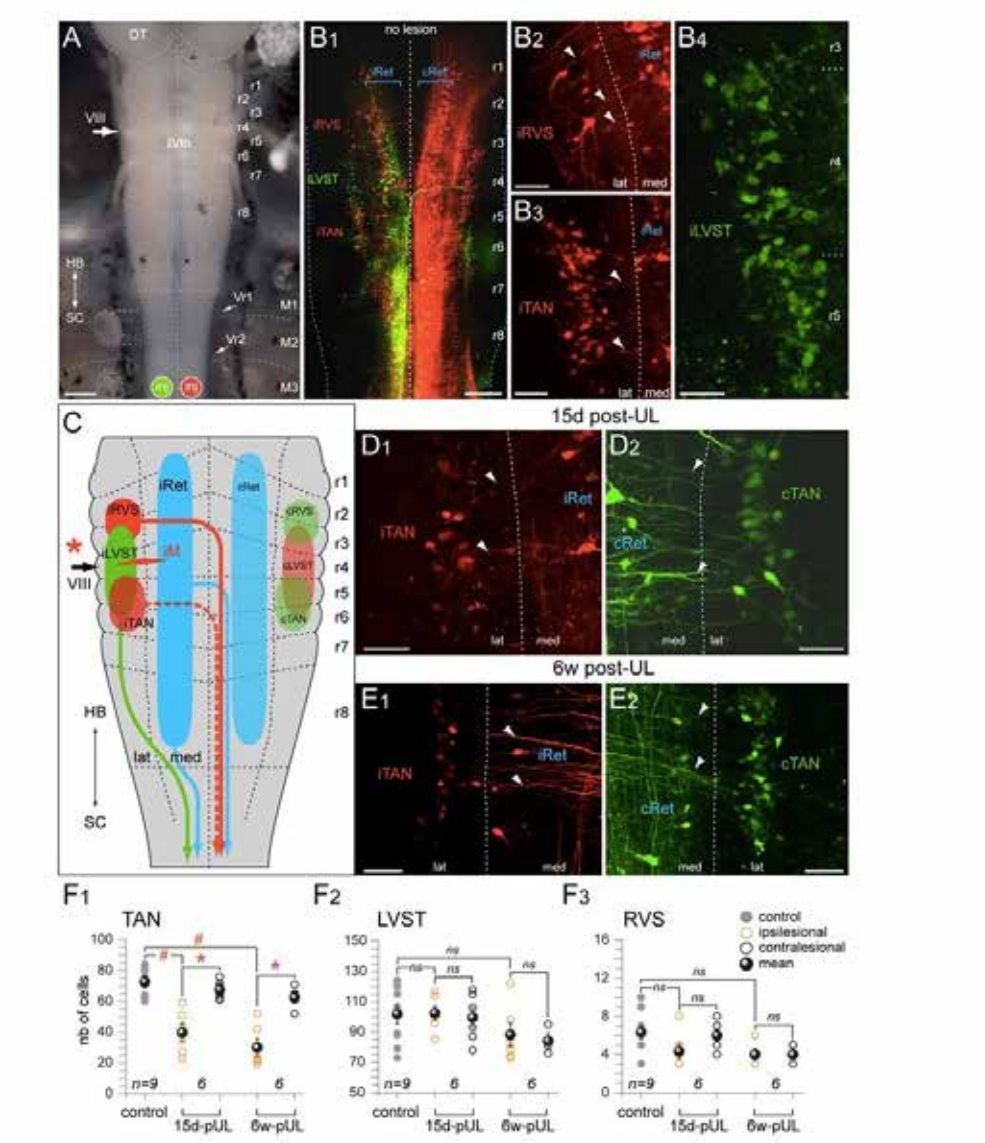
• Our third objective is to demonstrate that these databanks can be used in several ways for the benefit of the HMC under scrutiny: to train them to complex sensorimotor

Human idiopathic scoliosis is characterized by severe deformations of the spine and skeleton. The occurrence of vestibular-related deficits in these patients is well established but it is unclear whether a vestibular pathology is the common cause for the scoliotic syndrome and the gaze/posture deficits or if the latter behavioral deficits are a consequence of the scoliotic deformations. A possible vestibular origin was tested in the frog Xenopus laevis by unilateral removal of the labyrinthine endorgans at larval stages. After metamorphosis into young adult frogs, X-ray images and three-dimensional reconstructed micro-computer tomographic scans of the skeleton showed deformations similar to those of scoliotic patients. The skeletal distortions consisted of a curvature of the spine in the frontal and sagittal plane, a transverse rotation along the body axis and substantial deformations of all vertebrae. In terrestrial vertebrates, the initial postural syndrome after unilateral labyrinthectomy recovers over time and requires body weight-supporting limb proprioceptive information. In an aquatic environment, however, this information is absent. Hence, the lesion-induced asymmetric activity in descending spinal pathways and the resulting asymmetric muscular tonus persists. As a consequence the mostly cartilaginous skeleton of the frog tadpoles progressively deforms. Lack of limb proprioceptive signals in an aquatic environment is thus the element, which links the Xenopus model with human scoliosis because a comparable situation occurs during gestation in utero. A permanently imbalanced activity in descending locomotor/posture control pathways might be the common origin for the observed structural and behavioral deficits in humans as in the different animal models of scoliosis.

In order to test that hypothesis, peripheral vestibular lesions in larval Xenopus were used to reveal the morphophysiological alterations at the cellular and network levels. As a result, spinal motor nerves that were modulated by the previously intact side before UL remained permanently silent during natural vestibular stimulation after the lesion. In addition, retrograde tracing of descending pathways revealed a loss of vestibular neurons on the ipsilesional side with crossed vestibulospinal projections. This loss facilitated a general mass imbalance in descending premotor activity and a permanent asymmetric motor drive to the axial musculature. Therefore, we propose that the persistent asymmetric contraction of trunk muscles exerts a constant, uncompensated differential mechanical pull on bilateral skeletal elements that enforces a distortion of the soft cartilaginous skeletal element and bone shapes. This ultimately provokes severe scoliotic deformations during ontogenetic development similar to the human syndrome.



loss of vestibulospinal neurons after UL in stage 55–57 Xenopus tadpoles.



***A**, Photomicrograph of the hindbrain (HB) and rostral spinal cord (SC) depicting the sites of application of AlexaFluor 488 dextran (inj, green) and of Alexa Fluor 546 dextran (inj, red) to the left and right upper spinal cord, respectively, in controls and 15 d and 6weeks (6w) after UL on the left side.*

***B**, Confocal reconstruction of bilateral spinal-projecting neurons in the hindbrain of a control animal (**B**1, no lesion) illustrating the 3 major vestibulospinal cell groups with ipsilateral (i) or contralateral (c) axonal trajectories; higher magnification of retrogradely labeled neurons in the left vestibular nucleus that distinguish into a RVS (**B**2) and TAN (**B**3), both with crossed projections (labeled in red), respectively, and a third subgroup that gives rise to the uncrossed (labeled in green) lateral vestibulospinal tract (LVST, **B**4).*

***C**, Summary depicting the segmental organization along r1–r8 and axonal trajectories of the 3 major vestibulospinal subgroups, the Mauthner cell (M) and the bilateral, segmentally iterated reticulospinal neurons (iRet, cRet) on the ipsilesional side (red asterisk) and contralesional side.*

***D**, **E**, Confocal reconstructions of TAN neurons with midline-crossing descending axons on the ipsilesional side (iTAN;**D**1,**E**1) and contralesional side (cTAN;**D**2,**E**2) side 15 d (**D**) and*

*6 weeks (**E**, 6w) after UL; TAN neurons on the two sides of the brainstem were retrogradely labeled after unilateral application of two different tracers to the two sides of the upper spinal cord (**A**, inj).*

***F**, Numbers (mean_SE) of retrogradely labeled neurons in the TAN (**F**1), LVST (**F**2), and RVS (**F**3) subgroups of controls and on the ispilesional and contralesional side of (Figure legend continues.)*

Publications

F.M. Lambert, D. Malinvaud, J. Glaunès, C. Bergot, H. Straka, P.-P. Vidal 2009, Vestibular asymmetry as the cause of idiopathic scoliosis: a possible answer from Xenopus. J Neurosci. 2009 Oct 7;29(40):12477-83

F.M. Lambert, D. Malinvaud, M. Gratacap, H. Straka, P.-P. Vidal 2013, Neural plasticity in vestibulo-spinal pathways after unilateral labyrinthectomy as the origin for scoliotic deformations. J Neurosci. 2013 Apr 17;33(16):6845-6856

CHAPTER 4

Genetic studies

The existence of familial forms of Adolescent Idiopathic Scoliosis is long-standing and the genetic influences in the disease have been well documented. Many international teams aim at indentifying genetic markers and narrowing candidate genes associated with an increased risk for AIS. The study of rare variants in specific genes could also help for the development of diagnostic and therapeutic tools that identify asymptomatic children at risk of developing scoliosis
Research published in 2015 indicates the first gene directly identified with scoliosis and show how this mutation suggests that idiopathic scoliosis may primarily result from a brain dysfunction.

Deciphering the genetic causes of idiopathic scoliosis

Patrick Edery is a pediatrician and clinical geneticist, MD, PhD, who has set up a genetics department and a research group in Lyon, where he obtained a permanent position in 1998.
That year, he started to collect clinical data and blood samples from idiopathic scoliosis families with the valuable collaboration of the Massues Reeducation Center of Lyon. He first studied six French families using genetic tools (whole genome genotyping and linkage analysis) and found two chromosomal regions (5q and 3q), which co-segregated with the disease and reached statistical significance in one family with multiple affected patients (Edery P et al, Eur J Hum Genet 2011). This work was achieved thanks to the support of the Cotrel Foundation-Institut de France.

The idiopathic scoliosis-causing gene *POC5*, located on chromosome 5q, was recently identified and characterized by Patrick Edery and Florina Moldovan's teams. A large part of this work was accomplished by a post-doctoral scholar, Shunmoogum A Patten. Mutations in the *POC5* gene were found to contribute to the occurrence of idiopathic scoliosis in the large French family, in other French families (4/41 families included in the study) and in non-familial cases. When overexpressed in zebrafish, these human *POC5* mutations result in spine deformity similar to that observed in the patients (Patten SA, Moldovan F, Edery P. *J Clin Invest* 2015).
We also found that the *POC5* gene is strongly expressed in the zebrafish brain, suggesting that idiopathic scoliosis may primarily result from a brain dysfunction. In addition, the *POC5* gene is known to be involved in centriole maturation and primary cilia development, which suggests that a weakness in the establishment of body asymmetry may play a role in the occurrence of idiopathic scoliosis.

Introduction

Our research group, named "Genetics of neurodevelopment" and headed by Patrick Edery, was officially formed in 2011 within the Lyon Neuroscience Research Center.
We are currently applying for certification in 2016 as a new research team by INSERM, CNRS and the University Claude Bernard Lyon 1. Our main objectives are (*i*) to identify the genetic bases of developmental disorders such as idiopathic scoliosis and also intellectual disability, autism and epilepsy, (*ii*) to understand the pathophysiology of these diseases and (*iii*) to define possible prevention and therapeutic strategies. Our research activities are located both at the Lyon Est hospital and at the University Claude Bernard Lyon 1.
They combine fine clinical expertise and up-to date genetic technologies. Our group has published over 150 peer-reviewed articles since 2009.

Hypothesis and Methodology

Next, Patrick Edery's team planned to identify the idiopathic scoliosis-causing genes located within these two chromosomal regions and to prove their responsibility in the phenotype. A French-Canadian collaboration involving Patrick Edery and Florina Moldovan's research teams was undertaken in 2011 toward this aim, again thanks to the Cotrel Foundation-Institut de France. The methodologies used by these teams included clinical expertise, up-to date genetic technologies with next-generation sequencing and large data processing and zebrafish model.

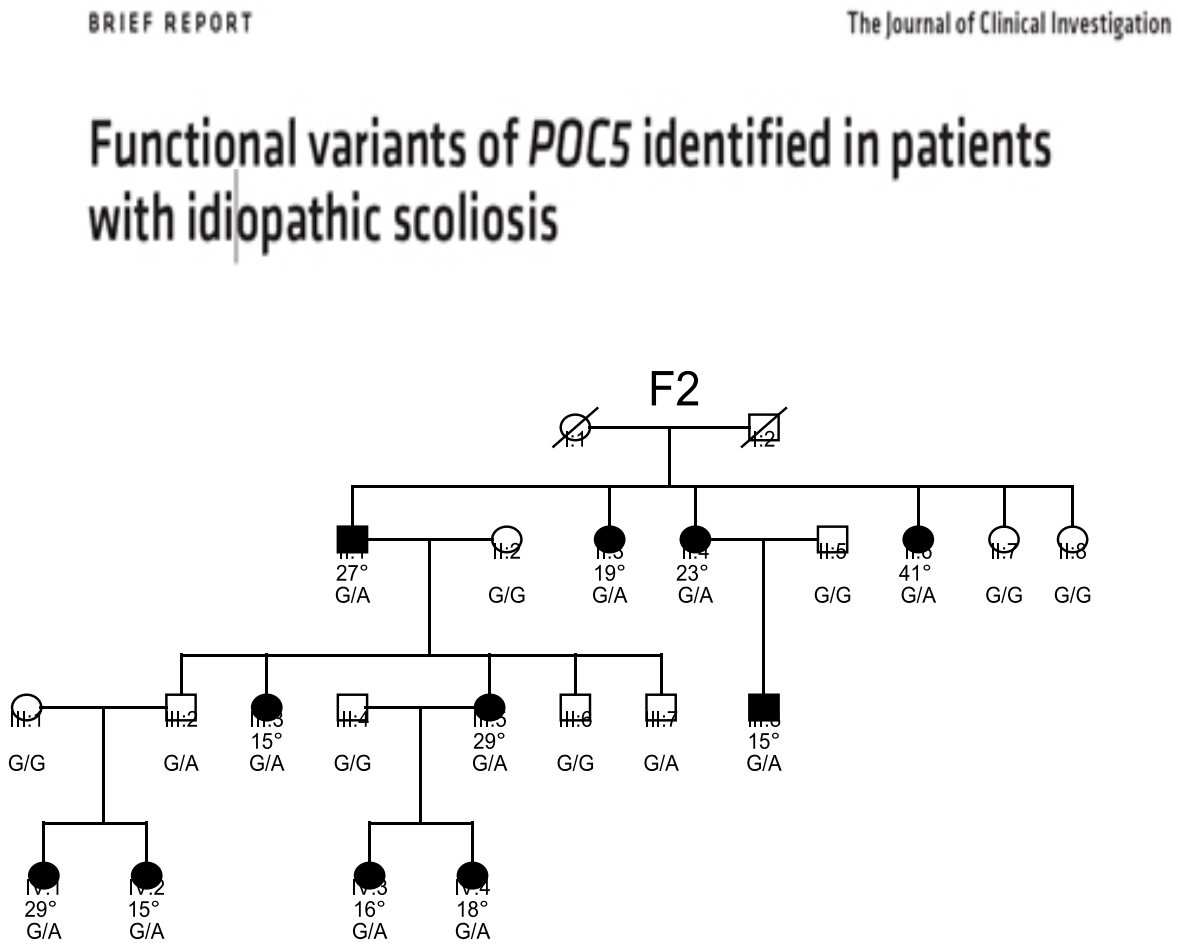
Perspectives

To the best of our knowledge, we identified the first gene shown not only to be genetically associated with idiopathic scoliosis, but also to result in this disease, when mutated.

The implications of our discovery in terms of medical care and prevention of idiopathic scoliosis in patients are currently investigated in Patrick Edery's team.

In the large French family, the *POC5* mutation is likely to result in idiopathic scoliosis through its interaction with another mutation located on chromosome 3q, which remains to be identified. A digenic heredity model should therefore be considered to explain the occurrence of the disease, at least in a subgroup of idiopathic scoliosis patients.

The role of *POC5* by itself in the disease remains to be investigated in large populations of various ethnicities. Our discoveries pave the way to deciphering the genetic bases of idiopathic scoliosis and understanding its pathophysiology, mandatory steps toward devising prevention and therapeutics.


BRIEF REPORT

The Journal of Clinical Investigation

Functional variants of *POC5* identified in patients with idiopathic scoliosis

Affected individual

POC5 G>A mutation

Genetic Linkage Analysis and Fine Mapping of Familial Idiopathic Scoliosis

Dr. Nancy Hadley Miller M.D., is an orthopedic surgeon, and is the principal investigator for this work. Our scoliosis research line has been directed to the etiology of idiopathic scoliosis. Over the course of time, this has involved multiple collaborations in the fields of clinical medicine, genetics, developmental biology and biostatistics. Early collaborators include Alexander Wilson, Ph.D. and Cristina Justice, Ph.D. at the National Institutes of Health (National Human Genome Research Institute), as well as Elizabeth Pugh, Ph.D. and Kim Doheny, Ph.D. at the Center for Inherited Disease Research (CIDR). Our work has also included international collaborations with Nelson Tang, M.D. and Dominique Rousié, M.D. Ph.D. Within the Miller laboratory, the work has been directed over time by Donna Schwab, M.S., Beth Marosy, M.S., Kandice Swindle B.A., and Erin Baschal, Ph.D. Additionally, students who have contributed to the work include Kris Alden, Nzeka Nzegwu, Marie-Hélène Roy-Gagnon, and Dana Behneman. This work has been completed at three institutions, Baylor College of Medicine in Houston, TX, Johns Hopkins University in Baltimore, MD, and the **University of Colorado Denver Anschutz Medical Campus**

Background

Idiopathic scoliosis occurs clinically in both sporadic and familial forms, but neither the genetic nor the molecular etiology for the disorder is currently understood. The discovery of idiopathic scoliosis genes and pathways would greatly enhance the overall understanding of the pathogenesis of this disorder and aid in the development of targeted diagnostics and therapeutics.

At the initiation of Dr. Miller's research directed to idiopathic scoliosis, the *FBN1* gene on chromosome 15, which encodes for fibrillin 1, had just been discovered as the genetic cause of Marfan syndrome. Marfan syndrome is a connective tissue disorder characterized by abnormalities of the cardiovascular, ocular, and musculoskeletal systems, including scoliosis. FBN1 is an essential component of connective tissues, specifically the elastic fiber system. The identification of a single gene as the cause for a multi-system disorder such as Marfan syndrome, which includes a scoliosis phenotype, led us to investigate potential FBN1 defects in patients with idiopathic scoliosis. This work was initiated at the University of Iowa and continued at Baylor College of Medicine with Dianna Milewicz, M.D. Analysis of FBN1 in elastic fibers from patients with scoliosis and age-matched controls resulted in the identification of FBN1 abnormalities in a select group of individuals with idiopathic scoliosis ; however, these defects did not seem to be the prevailing problem in the larger group of idiopathic scoliosis patients [1].

These results illuminated several problems with this experimental approach: studies could be investigating the incorrect tissue, investigating a correct tissue at the incorrect time in the disease process, or using the incorrect assay to measure the defect. These concerns, coupled with advancements in the field of genetics and the recognition of the familial nature of the disease, led us to pursue investigation of the genetic causes of idiopathic scoliosis.

Genetic studies in idiopathic scoliosis began with investigations of candidate genes, which were suspected to be involved in the disease process based on known functions. These studies capitalized on the genetic advances in microsatellite technology (STRPs), which allowed us to track STRP alleles through a pedigree. Carr et al. reported negative results for the collagen 1 and 2 genes in relation to idiopathic scoliosis [2]. Our studies, with Dianna Milewicz, M.D., investigated the extracellular matrix components FBN1, elastin, and type 1 collagen (COL1A2), but also yielded largely negative results [3]. This further established the need for studies that would investigate the genome as a whole, rather than focusing on specific candidate genes.

Hypothesis and Results

In the clinical setting, the familial nature of idiopathic scoliosis was well established, but the mode of inheritance for the disease was unclear [4-7]. At the same time, advancements in the fields of molecular and statistical genetics allowed for both candidate and genome-wide investigation of genes in families, through linkage analyses. Therefore, we began to establish a large collection of families that could be used for future genetic studies.

The hypothesis for our early studies was that the investigation of a large familial population, through linkage analyses, could elucidate the genes related to familial idiopathic scoliosis.

Our first project was a longitudinal 6 year project, devoted to linkage analyses in a large number of families affected with idiopathic scoliosis. An initial genome-wide scan was completed using a standard Weber STRP marker set [8, 9]. We completed this scan in our large study population of 202 idiopathic scoliosis families with at least two affected individuals (1198 individuals). This work led to the identification of seventeen candidate regions, four of which have been replicated in independent studies (**Table 1**) [10-13]. Identification of these candidate regions, although large, narrowed the potential candidates from thousands of genes across the genome to hundreds of genes located in specific regions.

Table 1: Replicated Candidate Linkage Regions [8, 9]

Chr.	Markers	Boundaries (Mb)	Length (Mb)	p-value*	Reference (replication)
9	D9S938 - D9S1838	101.36 -135.86	34.6	0.0005	[10]
16	D16S764 - D16S3253	16.61 - 54.57	37.96	0.0002	[11]
17	D17S1303 - D17S1293	11.06 - 32.71	21.65	0.0026	[12]
19	D19S591 - D19S714	3.03 - 15.59	12.56	0.02	[13]
*Most significant p-value obtained, genome build 33 (hg15)					

The next goal was to further reduce the number of candidates by narrowing the candidate regions identified above. The detailed clinical characterization of the current study population allowed for segregation of homogenous groups of families that could be genetically meaningful. The initial study population was stratified in multiple ways:

1) consideration of familial idiopathic scoliosis as a qualitative versus a quantitative trait;
2) considering potential differing modes of heritability (autosomal dominant and/or X-linked dominant), and 3) subgrouping families *a priori* based on clinical findings. Each strategy successfully resulted in the identification of critical loci that underwent verification through select high-density SNP linkage and association analyses (**Table 2**) [8, 14-17].

Table 2: Phenotypic Subgroup Linkage Analyses

Phenotypic Family Subgroup	Sample Type/Analysis	Chromosomal Region(s)	p value (range)	Reference
X-linked dominant	51 families/linkage	Xq23	0.0014	[8]
Males with severe scoliosis requiring surgery	72 families/linkage	19p13	0.01356	[14]
Kyphoscoliosis	7 families/linkage, association	5q13 13q32	0.0017 0.0001	[15]
Males with >30° curvature	17 families/ linkage	17p11	0.0003	[16]
Triple curves	5 families/linkage	6q16 10q23	$2x10^{-10}$ to $1x10^{-6}$	[17]

Future directions

These studies, in conjunction with recent genome-wide studies [18-21], indicate that the underlying genetics for both familial and sporadic idiopathic scoliosis is complex and likely due to multiple genes, as it has been difficult to pinpoint genes or variants that are important for all individuals with idiopathic scoliosis, even within phenotypic subgroups. Advances in Next-Generation sequencing have allowed for cost-effective exome sequencing (sequencing the coding regions in each individual) for multiple individuals affected by a disease, allowing for the identification and analysis of rare genetic variants (frequency less than 1%). Exome sequencing has been successful thus far in idiopathic scoliosis, as studies have identified rare variants in genes (*FBN1*, *FBN2*, *POC5*, and *HSPG2*) that may contribute to the etiology of idiopathic scoliosis [22-24]. Additionally, these variants may work with variants in other genes to modify the effect(s) and result in clinical/identifiable disease (*i.e.*, variants in multiple genes may be required for a larger/progressive curvature).

The marked heterogeneity in genetic causes for idiopathic scoliosis emphasizes the need for collaborative work. We have been involved in the development of the International Consortium for Scoliosis Genetics (ICSG), a consortium directed to understanding the genetic etiology of this disorder. This is an international collaborative effort that includes clinicians and scientists from multiple disciplines. The integration of these individuals from both the clinical world and basic science will allow for a better understanding of the phenotype in relation to the genetic underpinnings and molecular pathways that lead to the development of idiopathic scoliosis.

References

1. Hadley-Miller N, Mims B, Milewicz DM. The potential role of the elastic fiber system in adolescent idiopathic scoliosis. J Bone Joint Surg Am. Aug 1994;76(8):1193-1206.

2. Carr AJ, Ogilvie DJ, Wordsworth BP, Priestly LM, Smith R, Sykes B. Segregation of structural collagen genes in adolescent idiopathic scoliosis. Clin Orthop Relat Res. Jan 1992(274):305-310.

3. Miller NH, Mims B, Child A, Milewicz DM, Sponseller P, Blanton SH. Genetic analysis of structural elastic fiber and collagen genes in familial adolescent idiopathic scoliosis. J Orthop Res. Nov 1996;14(6):994-999.

4. Bonaiti C, Feingold J, Briard ML, Lapeyre F, Rigault P, Guivarch J. [Genetics of idiopathic scoliosis]. Helv Paediatr Acta. Oct 1976;31(3):229-240.

5. Czeizel A, Bellyei A, Barta O, Magda T, Molnar L. Genetics of adolescent idiopathic scoliosis. J Med Genet. Dec 1978;15(6):424-427.

6. Riseborough EJ, Wynne-Davies R. A genetic survey of idiopathic scoliosis in Boston, Massachusetts. J Bone Joint Surg Am. Jul 1973;55(5):974-982.

7. Cowell HR, Hall JN, MacEwen GD. Genetic aspects of idiopathic scoliosis. A Nicholas Andry Award essay, 1970. Clin Orthop Relat Res. Jul-Aug 1972;86:121-131.

8. Justice CM, Miller NH, Marosy B, Zhang J, Wilson AF. Familial idiopathic scoliosis: evidence of an X-linked susceptibility locus. Spine. Mar 15 2003;28(6):589-594.

9. Miller NH, Justice CM, Marosy B, Doheny KF, Pugh E, Zhang J, Dietz HC, 3rd, Wilson AF. Identification of candidate regions for familial idiopathic scoliosis. Spine. May 15 2005;30(10):1181-1187.

10. Ocaka L, Zhao C, Reed JA, Ebenezer ND, Brice G, Morley T, Mehta M, O'Dowd J, Weber JL, Hardcastle AJ, Child AH. Assignment of two loci for autosomal dominant adolescent idiopathic scoliosis to chromosomes 9q31.2-q34.2 and 17q25.3-qtel. J Med Genet. Feb 2008;45(2):87-92.

11. Salehi LB, Mangino M, De Serio S, De Cicco D, Capon F, Semprini S, Pizzuti A, Novelli G, Dallapiccola B. Assignment of a locus for autosomal dominant idiopathic scoliosis (IS) to human chromosome 17p11. Hum Genet. Oct 2002;111(4-5):401-404.

12. Morcuende J. In: Miller NH, ed. Personal Communication ed2008.

13. Chan V, Fong GC, Luk KD, Yip B, Lee MK, Wong MS, Lu DD, Chan TK. A genetic locus for

adolescent idiopathic scoliosis linked to chromosome 19p13.3. Am J Hum Genet. Aug 2002;71(2):401-406.

14. Alden KJ, Marosy B, Nzegwu N, Justice CM, Wilson AF, Miller NH. Idiopathic scoliosis: identification of candidate regions on chromosome 19p13. Spine. Jul 15 2006;31(16):1815-1819.

15. Miller NH, Marosy B, Justice CM, Novak SM, Tang EY, Boyce P, Pettengil J, Doheny KF, Pugh EW, Wilson AF. Linkage analysis of genetic loci for kyphoscoliosis on chromosomes 5p13, 13q13.3, and 13q32. Am J Med Genet A. May 15 2006;140(10):1059-1068.

16. Clough M, Justice CM, Marosy B, Miller NH. Males with familial idiopathic scoliosis: a distinct phenotypic subgroup. Spine (Phila Pa 1976). Jan 15 2010;35(2):162-168.

17. Marosy B, Justice C, Vu C, Zorn A, Nzegwu N, Wilson A, Miller N. Identification of Susceptibility Loci for Scoliosis in FIS Families with Triple Curves. AJMG. 2010.

18. Kou I, Takahashi Y, Johnson TA, Takahashi A, Guo L, Dai J, Qiu X, Sharma S, Takimoto A, Ogura Y, Jiang H, Yan H, Kono K, Kawakami N, Uno K, Ito M, Minami S, Yanagida H, Taneichi H, Hosono N, Tsuji T, Suzuki T, Sudo H, Kotani T, Yonezawa I, Londono D, Gordon D, Herring JA, Watanabe K, Chiba K, Kamatani N, Jiang Q, Hiraki Y, Kubo M, Toyama Y, Tsunoda T, Wise CA, Qiu Y, Shukunami C, Matsumoto M, Ikegawa S. Genetic variants in GPR126 are associated with adolescent idiopathic scoliosis. Nat Genet. Jun 2013;45(6):676-679.

19. Takahashi Y, Kou I, Takahashi A, Johnson TA, Kono K, Kawakami N, Uno K, Ito M, Minami S, Yanagida H, Taneichi H, Tsuji T, Suzuki T, Sudo H, Kotani T, Watanabe K, Chiba K, Hosono N, Kamatani N, Tsunoda T, Toyama Y, Kubo M, Matsumoto M, Ikegawa S. A genome-wide association study identifies common variants near LBX1 associated with adolescent idiopathic scoliosis. Nat Genet. Dec 2011;43(12):1237-1240.

20. Sharma S, Gao X, Londono D, Devroy SE, Mauldin KN, Frankel JT, Brandon JM, Zhang D, Li QZ, Dobbs MB, Gurnett CA, Grant SF, Hakonarson H, Dormans JP, Herring JA, Gordon D, Wise CA. Genome-wide association studies of adolescent idiopathic scoliosis suggest candidate susceptibility genes. Hum Mol Genet. Apr 1 2011;20(7):1456-1466.

21. Londono D, Kou I, Johnson TA, Sharma S, Ogura Y, Tsunoda T, Takahashi A, Matsumoto M, Herring JA, Lam TP, Wang X, Tam EM, Song YQ, Fan YH, Chan D, Cheah KS, Qiu X, Jiang H, Huang D, Group TIC, International Consortium for Scoliosis G, Su P, Sham P, Cheung KM, Luk KD, Gordon D, Qiu Y, Cheng J, Tang N, Ikegawa S, Wise CA. A meta-analysis identifies adolescent idiopathic scoliosis association with LBX1 locus in multiple ethnic groups. J Med Genet. Jun 2014;51(6):401-406.

22. Buchan JG, Alvarado DM, Haller GE, Cruchaga C, Harms MB, Zhang T, Willing MC, Grange DK, Braverman AC, Miller NH, Morcuende JA, Tang NL, Lam TP, Ng BK, Cheng JC, Dobbs MB, Gurnett CA. Rare variants in FBN1 and FBN2 are associated with severe adolescent idiopathic scoliosis. Hum Mol Genet. May 15 2014.

23. Baschal EE, Wethey CI, Swindle K, Baschal RM, Gowan K, Tang NL, Alvarado DM, Haller GE, Dobbs MB, Taylor MR, Gurnett CA, Jones KL, Miller NH. Exome Sequencing Identifies a Rare HSPG2 Variant Associated with Familial Idiopathic Scoliosis. G3 (Bethesda). 2014;5(2):167-174.

24. Patten SA, Margaritte-Jeannin P, Bernard JC, Alix E, Labalme A, Besson A, Girard SL, Fendri K, Fraisse N, Biot B, Poizat C, Campan-Fournier A, Abelin-Genevois K, Cunin V, Zaouter C, Liao M, Lamy R, Lesca G, Menassa R, Marcaillou C, Letexier M, Sanlaville D, Berard J, Rouleau GA, Clerget-Darpoux F, Drapeau P, Moldovan F, Edery P. Functional variants of POC5 identified in patients with idiopathic scoliosis. J Clin Invest. Feb 2 2015.

Publications Resulting from Funding from Fondation Yves Cotrel:

1. Familial idiopathic scoliosis: Evidence of an X-linked susceptibility. Miller NH, Justice CM, Marosy B, Zhang J, Wilson AF. Spine. 28(6):589-94. 2003.

2. Identification of Candidate Regions for Familial Idiopathic Scoliosis. Miller NH, Justice CM, Marosy B, Doheny KF, Pugh E, Zhang J, Dietz HC, Wilson AF, Spine. 30(10):1181-1187. 2005.

3. Idiopathic scoliosis: identification of candidate regions on chromosome 19p13. Alden KJ, Marosy B, Nzegwu N, Justice CM, Wilson AF, Miller NH: Spine. 31(16):1815-1819. 2006.

4. Lack of Association between the Aggrecan Gene and Familial Idiopathic Scoliosis. Marosy B, Justice C, Nzegwu N, Kumar G, Wilson AF, Miller NH. Spine. 31(13):1420-1425, 2006.

5. Linkage Analysis of Genetic Loci for Kyphoscoliosis on Chromosomes 5p13, 13q13.3, and 13q32. Miller NH, Marosy B, Justice CM, Novak SM, Tang EY, Boyce P, Pettengil J, Doheny KF, Pugh EW, Wilson AF. Am J Med Genet A. 140(10):1059-68. 2006.

6. Identification of susceptibility loci for scoliosis in FIS families with triple curves. Marosy B, Justice CM, Vu C, Zorn A, Nzegwu N, Wilson AF, Miller NH. Am J Med Genet A. 152A(4):846-55. 2010.

7. Males with Familial Idiopathic Scoliosis: a Distinct Phonotypic Subgroup. Clough M, Justice CM, Marosy B, Miller NH. Spine. 35(2):162-8. 2010.

8. Intra-Familial Tests of Association between Familial Idiopathic Scoliosis and Linked Regions on 9q31.3 q34.3 and 16p12.3 q22.2. Miller NH, Justice CM, Marosy B, Swindle K, Kim Y, Roy-Gagnon M, Sung H, Behneman D, Doheny KF, Pugh E, Wilson AF. Hum Hered. 74(1):36-44. 2012.

9. CHD7 gene polymorphisms and familial idiopathic scoliosis. Tilley MK, Justice CM, Swindle K, Marosy B, Wilson AF, Miller NH. Spine (Phila Pa 1976). 38(22):E1432-6. 2013.

Localization and analysis of candidate genes for idiopathic scoliosis

Carol A. Wise, PhD
Texas Scottish Rite Hospital for Children, University of Texas Southwestern Medical Center

Our research centers on discovering genes that increase susceptibility to idiopathic scoliosis, and on understanding the functions of those genes. Almost a century of literature describes familial forms of IS, particularly adolescent-onset IS (AIS) (1). The observation that AIS clusters in families provided initial evidence of genetic influences in the disease (2-4). Twin studies have consistently shown higher concordance in monozygotes compared to dizygotes, pointing to heritable factors (5, 6). AIS sibling risk and heritability estimates also support significant genetic contributions. Sibling recurrence risks greater than 17% have been measured in both Asian and European populations (compared to the population risk of ~3%) (7). Heritability estimates for AIS are quite high, suggesting that greater than 80% of disease risk is due to genetic factors, with overall complex (multi-genic) inheritance (7-9). Taking the evidence altogether, AIS in most cases can be described as a complex, multigenic disease, controlled by many genes.

Gene Discovery for Human AIS

The significant heritability of IS has motivated the search for genetic risk factors that can explain disease liability. One approach has been unbiased, genome-wide searches, so-called genome-wide association studies or GWAS, to define disease loci. GWAS involves genotyping a high density (typically e»350,000) of common single nucleotide polymorphisms (SNPs) that span the entire genome. Statistical comparisons between cases and controls for each genotyped SNP identify those that associated with disease state, with the expectation that true associations will cluster in the genome, effectively mapping genetic risk factors. Due to the many tests involved, that is, the many SNPs that are genotyped, a threshold $P < 3x10^{-8}$ is required to declare a significant association with the condition, i.e. the disease, that is tested. We performed the first GWAS of AIS in a cohort of 419 families. This study generated strongest signals near the *CHL1* gene, a cell adhesion protein involved in axon guidance (10). Deficiencies in commissural axon guidance mechanisms are known to cause scoliosis, as evidenced by the Mendelian disorder Horizontal Gaze Palsy with Progressive Scoliosis (HGPPS, MIM #607313). HGPPS is caused by recessively inherited mutations in the *ROBO3* gene that, like *CHL1,* is an axon guidance gene (10). In a second study, our group led a meta-analysis of the *LBX1* locus in AIS, producing strong evidence for this susceptibility locus, with a highly significant association ($P = 1.22\times10^{-43}$) for SNP rs11190870 (11). The LBX1 protein is involved in muscle and nerve specification (12). Targeted inactivation in the mouse has shown that this gene is necessary for lateral migration of muscle precursors into the limbs (13) and for proper development of cells of the dorsal spinal cord (14). In a third study, we collaborated with Dr. Shiro Ikegawa in Japan and replicated association ($P = 1.27x10^{-14}$) with SNPs in the *GPR126* gene (15). *GPR126*, a member of the adhesion G protein-coupled receptor (GPCR) family (16). In zebrafish, *gpr126* is required for Schwann cell myelination (17). *Gpr126* null mutant mice also show delayed axonal sorting by Schwann cells and severe neurologic deficits (18, 19). In a fourth and recent study, by an expanded two-stage GWAS and replication in AIS cohorts (total N= 6,178) and follow-up functional genomics, we recently demonstrated that mutations disrupting an enhancer of the transcription factor *PAX1* were strongly

associated with AIS in females. This is the first study to identify AIS causal mutations, and the first to identify genetic risk factors underlying its sexual dimorphism. PAX1 is a well-described, key player in early somite development. This discovery raises the exciting possibility that PAX1 also affects later spinal development, i.e. after the somites are patterned, possibly via affects on paravertebral muscle where we showed that it is expressed post-somitogenesis (20). Additional GWAS with sufficient power to detect new AIS risk loci are clearly necessary, but the design of such studies is a challenge. Much larger cohorts, on the order of tens of thousands, are needed. Additionally, reducing genetic heterogeneity by refined phenotyping is also perceived as an unfulfilled goal in the AIS field. These efforts arguably can only be performed through research consortia. Consequently we formed the International Consortium for Scoliosis Genetics (ICSG) 2012 (**Figure 1**). In 2014 ICSG produced the first AIS large-scale genetic meta-analysis, the study of the *LBX1* locus described above (11). Ongoing consortium efforts also include genome-wide meta-analysis of existing datasets and organizational efforts to support the creation of much larger cohorts, on the order of >10,000 patients. Prospective, detailed, and standardized phenotyping of existing cohorts is a key goal of ICSG in AIS research.

Gene Discovery for Early Onset IS (EOIS)

Children with early onset scoliosis can pose a significant and challenging clinical problem, as they are at risk for pulmonary compromise as well as other growth disturbances (21). The pathogenesis of EOS is heterogeneous, with roughly one-third of surgical cases having no identifiable diagnosis, so called early onset "idiopathic" scoliosis (EOIS). Unlike later onset AIS, unexplained EOIS rarely presents with positive family history of scoliosis and may affect boys more than girls (22). We hypothesized that EOIS could arise from rare *de novo* mutations, in other words, mutations that are absent in the parents and the general population but present in the affected offspring.

We also hypothesized that such mutations are likely to be heterogeneous, that is, to correspond to many different causal genes, reflecting the clinical heterogeneity observed in this population.

To test this hypothesis we assessed a cohort of 24 children with unexplained EOS using microarray-based genotyping. Our results identified *de novo* mutations in three children, two that were clearly disease-causing and clarified an underlying diagnosis (23).

Summary. We have conducted large, collaborative, population-based studies that have identified genetic markers associated with increased risk for AIS (**Table 1**). Markers near PAX1 may alter its regulation, an effect apparently specific to female AIS. The known roles of candidate genes in nerve and muscle biology implicate these tissues in AIS, but more research is needed to understand these processes in the spine. Our studies of EOIS underscore the utility of updated genetic evaluations including high-density microarray-based genotyping in patients with unexplained EOS, even where prior genetic studies were negative. These data also suggest the intriguing possibility that other *de novo* mutations detectable by whole genome sequencing, as well as epigenetic effects, await discovery in the EOIS population.

Locus	Region	Marker	Method	LOD	P value (OR)	Candidate gene	Reference
-	3p26.3	rs10510181	GWAS	-	2.58×10^{-8}	*CHL1*	10
-	10q24.31	rs11190870	GWAS	-	1.24×10^{-19} (1.56)	*LBX1*	11,12
-	6q24.1	rs6570507	GWAS	-	1.27×10^{-14} (1.27)	*GPR126*	15
-	20p11.22	Rs6137473	GWAS	-	2.15×10^{-10}	*PAX1*	20

Table 1. Summary of GWAS-based gene discovery

References

1. Staub HA. Eine skoliotikerfamilie. Ein Beitrag zur Frage der kongenitalen Skoliose und der Hereditat der Skoliosen. Z Orthop Chir 1922;43(1).

2. Garland HG. Hereditary Scoliosis. British medical journal. 1934;1(3816):328.

3. Filho NA, Thompson, M.W. . Genetic studies in scoliosis. J Bone Joint Surg Am 1971;53(199).

4. Bell M, Teebi AS. Autosomal dominant idiopathic scoliosis? American journal of medical genetics. 1995;55(1):112.

5. Kesling KL, Reinker KA. Scoliosis in twins. A meta-analysis of the literature and report of six cases. Spine (Phila Pa 1976). 1997;22(17):2009-14; discussion 15.

6. Andersen MO, Thomsen K, Kyvik KO. Adolescent idiopathic scoliosis in twins: a population-based survey. Spine (Phila Pa 1976). 2007;32(8):927-30.

7. Tang NL, Yeung HY, Hung VW, Di Liao C, Lam TP, Yeung HM, et al. Genetic epidemiology and heritability of AIS: A study of 415 Chinese female patients. J Orthop Res. 2012;30(9):1464-9.

8. Riseborough EJ, Wynne-Davies R. A genetic survey of idiopathic scoliosis in Boston, Massachusetts. J Bone Joint Surg Am. 1973;55(5):974-82.

9. Ward K, Ogilvie J, Argyle V, Nelson L, Meade M, Braun J, et al. Polygenic inheritance of adolescent idiopathic scoliosis: a study of extended families in Utah. Am J Med Genet A. 2010;152A(5):1178-88.

10. Sharma S, Gao X, Londono D, Devroy SE, Mauldin KN, Frankel JT, et al. Genome-wide association studies of adolescent idiopathic scoliosis suggest candidate susceptibility genes. Hum Mol Genet. 2011;20(7):1456-66.

11. Londono D, Kou I, Johnson TA, Sharma S, Ogura Y, Tsunoda T, et al. A meta-analysis identifies adolescent idiopathic scoliosis association with LBX1 locus in multiple ethnic groups. Journal of medical genetics. 2014;51(6):401-6.

12. Takahashi Y, Kou I, Takahashi A, Johnson TA, Kono K, Kawakami N, et al. A genome-wide association study identifies common variants near LBX1 associated with adolescent idiopathic scoliosis. Nat Genet. 2011;43(12):1237-40.

13. Gross MK, Moran-Rivard L, Velasquez T, Nakatsu MN, Jagla K, Goulding M. Lbx1 is required for muscle precursor migration along a lateral pathway into the limb. Development. 2000;127(2):413-24.

14. Gross MK, Dottori M, Goulding M. Lbx1 specifies somatosensory association interneurons in the dorsal spinal cord. Neuron. 2002;34(4):535-49.

15. Kou I, Takahashi Y, Johnson TA, Takahashi A, Guo L, Dai J, et al. Genetic variants in GPR126 are associated with adolescent idiopathic scoliosis. Nat Genet. 2013;45(6):676-9.

16. Langenhan T, Aust G, Hamann J. Sticky signaling—adhesion class g protein-coupled receptors take the stage. Science signaling. 2013;6(276):re3.

17. Monk KR, Naylor SG, Glenn TD, Mercurio S, Perlin JR, Dominguez C, et al. A G protein-coupled receptor is essential for Schwann cells to initiate myelination. Science. 2009;325(5946):1402-5.

18. Monk KR, Oshima K, Jors S, Heller S, Talbot WS. Gpr126 is essential for peripheral nerve development and myelination in mammals. Development. 2011;138(13):2673-80.

19. Waller-Evans H, Promel S, Langenhan T, Dixon J, Zahn D, Colledge WH, et al. The orphan adhesion-GPCR GPR126 is required for embryonic development in the mouse. PloS one. 2010;5(11):e14047.

20. Sharma S LD, Eckalbar WL, Gao X, Zhang D, Mauldin K, Kou I, Atsushi Takahashi A, Matsumoto M, Kamiya N, Murphy KK, Cornelia R, TSRHC Scoliosis Clinical Group, Japan Scoliosis Clinical Research Group, Herring JA, Burns D, Ahituv N, Ikegawa S, Gordon D, Wise CA. A PAX1 enhancer locus is associated with susceptibility to idiopathic scoliosis in females. Nature Communications. 2015;in press.

21. Herring J, editor. Tachdjian's Pediatric Orthopaedics. 5th ed. Philadelphia: WB Saunders; 2013.

22. Wynne-Davies R. Infantile Idiopathic Scoliosis: Causative Factors, Particularly in the First Six Months of Life. Journal of bone and Joint Sugery. 1975;57-B(2):138-41.

23. Gao X GG, Rathjen K , Johnston C, Sparagana S, Rios J, Wise CA. Genomic analyses of patients with unexplained early onset scoliosis. Spine Deformity. 2014;2(5):324-32.

Study of molecular and genetic determinants of adolescent idiopathic scoliosis: an international collaboration

Dr. Alain Moreau, (PhD, Montreal, Canada)

Dr. Hubert Labelle (MD, Montreal, Canada) is a clinical-scientist and orthopaedic spine surgeon who ha been collaborating with Dr. Moreau since 2001. He will assist Dr. Moreau in all clinical aspects related to spinal deformity progression and he will coordinate the clinical evaluation of the French-Canadian cohort.

Dr. Jack. C. Cheng (MD, Hong Kong, China) is a clinical-scientist internationally recognized for his pioneer works on AIS etiology, and who has been collaborating with Dr. Moreau since 2008. He will assist Dr. Moreau in the clinical validation of the Chinese AIS cohort (Hong Kong, China).

This research project demonstrated in patients with adolescent idiopathic scoliosis (AIS) the presence of rare variants in the regions that control the activity of genes as well as other variants that affect the coding regions (exons) that are responsible for the development of certain proteins. We selected 25 genes exhibiting rare variants in the French-Canadian population and are currently in the large-scale validation step in different populations. In parallel, we have demonstrated that variants previously identified in a first genetic test marketed under the name Scoliscore, had no predictive or clinical relevance in our European descent population, also confirming two other studies performed with Asian populations (Japanese and Chinese). The novel genes and variants selected as part of our study will help for the development of diagnostic and prognostic tools to help clinicians to identify asymptomatic children at risk of developing scoliosis and offer new therapeutic approaches for those affected.

Background

Although adolescent idiopathic scoliosis (AIS), the most common form of scoliosis, is a disease affecting a large number of young adolescents (0.2%-6% of the population), its genetic causes remain unclear[1;2]. Evidence of genetic influences for AIS comes from a number of sources, including epidemiologic studies of family history and family clustering, twin studies, and exploration of rare genetic disorders[3-6]. These studies have also highlighted potential differences in the degree of AIS heritability. This suggests a high level of heterogeneity in the nature of the encoded susceptibility genes, which is reflected by the increasing number of loci identified in AIS linkage studies and discrepancies among these studies. The pathophysiological relevance of susceptibility loci has yet to be confirmed, illustrating our incomplete knowledge of the genetics and biology of AIS. In fact, familial scoliosis represents only 15% of all AIS cases. In this context, classical genetic approaches to AIS, which focus only on familial cases, are very limited in the extent to which they are informative about the etiopathogenesis of this disease. Idiopathic scoliosis is an end phenotype that can be a consequence of multiple genetic defects in conserved biological pathways that are involved in the maintenance of spinal integrity and stability. Identification of genes and biological pathways involved in idiopathic scoliosis is critical for understanding its etiopathogenesis and better studying the gene-environment interactions in modulating the incidence and severity of the phenotype.

Although a genetic basis is acknowledged from familial studies, the mode of inheritance appears

to be heterogeneous. Reports of AIS inheritance include autosomal dominant, autosomal recessive, and multifactorial (reviewed by Gorman, et al., 2014)[7]. The majority of genetic studies for AIS prior to 2010 are candidate gene based, with candidates chosen from hypotheses generated by clinical observations. Most of these genes are unconfirmed or have mixed published results, depending on the design of the study and/or populations tested (reviewed by Gorman et al. 2012)[8]. Taken as a whole, there has been very little success in identifying genetic factors for complex multifactorial diseases before the advent of GWAS, an approach that is based on the "Common Disease Common Variant" model. Considering the lack of success from AIS hypothesis driven genetic studies and the prevalence of the disease, the application of GWAS to AIS is logical, with the expectation that a number of modest effect variants that associate with predisposition and/or severity would be identified.

The first GWAS of AIS was conducted by Sharma et al. (2011)[9] who surveyed 419 trio-families (affected children and their parents) in Utah to generate a list of 100 significantly associated SNPs that were then combined with three independent replication studies having a total of 3431 individuals. The most significant SNP (rs10510181) had an odds ratio OR=1.37 (95% confidence interval: CI=1.20–1.58, p=8.22e-7). The results suggested that CHL1, a member of the L1 gene family of neural cell adhesion molecules, might be associated with susceptibility. The authors then surveyed variants significantly associated with other genes involved in the axon guidance pathway: DSCAM (p=2.26e-5 for rs2222973) and CNTNAP2 (p=6.20e-5 for rs11770843) but concluded that larger cohorts are necessary to verify their findings and to identify additional susceptibility loci. A recent study using a Han Chinese population did not replicate the association between CHL1 and AIS[10], suggesting that either the risk is population specific or type I error. Takahashi et al. (2011)[11] used 1,376 female Japanese with AIS and 11,297 female controls to demonstrate an association to 3 variants (combined P = 1.24 × 10(-19); odds ratio (OR) = 1.56) near LBX1, a transcription factor required for the development of inhibitory interneurons in the dorsal horn of the spinal cord as well as migration and further development of hypaxial muscle precursor cells for limb muscles.

The association was independently replicated in two Chinese Han populations.[12,13] Recently, Kou et al. (2013)[14] demonstrated a modest association (P = 2.25 × 10(-10); odds ratio (OR) = 1.28) between a variant in GPR126 and 1819 Japanese AIS cases (vs. 25,939 control). In sum, these recent GWAS suggest two, possibly three, genes that pose a minor risk for AIS susceptibility.

Hypothesis

Increased vulnerability for AIS could be due to sustained exposure to high levels of OPN and/or a combination of predisposition genes enhancing the effect of OPN by their up/down-regulated gene expression patterns. Since girls are more affected in number and severity, sexual hormones could exacerbate such pre-existing conditions through a crosstalk with the OPN signal transduction pathway.

Methodology : Our functional cell based assay led us to demonstrate a systemic and differential dysfunction of Gi-coupled receptor signaling in osteoblasts and other cell types isolated from AIS patients, leading to their stratification into three endophenotypes. The heredity of these endophenotypes has been confirmed by testing affected family members. Genomic data was collected from the French-Canadian population, using the Illumina HumanOmni 2.5M BeadChip. The affordability of next generation sequencing technologies has allowed us to shift our focus from the identification of modest effect risk alleles to genes involved in the etiology of the disease. We completed exome sequencing of 48 severe AIS cases vs. 62 matched controls in our French-Canadian AIS cohort to identify a short list of candidate genes. Our choice to use severe cases only ensures us that if AIS is polygenic and has an additive component, our sample will be enriched for genetic factors. As an extra quality control procedure to ensure the reliability of our SNV data, we compared the SNV (SNP) calls between our WES data and PCR amplified-Sanger sequencing replications for 100 random positions. The WES analysis reports SNVs findings with their associated phredscores for mapping quality of the reads (MQ), genotype quality of the SNVs (GQ) and the quality of the variant allele itself (alt qual). Thus, a cross-comparison of Sanger and WES results for 100 variants allowed us to define the most accurate threshold to filter out potential artefacts. SNVs from WES having GQ e» 80, alt qual e» 50 and MQ e» 20 have 90% of chances to be confirmed by Sanger sequencing (this stands for SOLiD 5 technology only). Subsequent analyses

which included a collapsing method to detect the association/accumulation of rare variants in our AIS patient cohort were performed. Finally, our functional classification led us also to determine their molecular profiles using the Gene Chip human genome array Affymetrix (HuU133 1.0ST) and a screening for alterations in expression profile of specific candidate genes performed under stringent conditions (± 3-fold expression change and 5% FDR).

Results obtained

Genome-Wide Association Study (GWAS).

Understanding that AIS is a common complex genetic disease, our initial genetic inquiry was a GWAS to examine the French-Canadian cohort (667 AIS cases vs. 904 matched controls) for shared common genetic variants. We used the Illumina 2.5-8 BeadChip, representing the highest resolution GWAS in the AIS field to date, with over 1.4 million variants. The design of this study was novel in that we used the biochemical endophenotypes to partition the cases, expecting that we would have increased power to detect variants in our relatively small population. Our analyses considered predisposing variants (case vs control) as well as variants associated with curve severity, and the biochemical endophenotypes. We did not identify significant DNA variants when we filtered our data for MAF < 2.5% (Minor Allele Frequency). Suggestive results will be validated in future studies that include a large confirmation cohort of French-Canadian patients (recruitment ongoing), as well as collaborations for meta-analyses. Of note, we recently published a paper showing that none of the SNPs used in ScoliScore™ were associated with AIS curve progression or curve occurrence in the French-Canadian population. We evaluated 52 SNPs in severe patients by comparing risk allele frequencies with those in non-severe patients and with those in control individuals. There was no significant difference between the severe group and the non-severe group or between the severe group and the control group. Although the 52 SNPs studied here were previously associated with curve progression in an American population of European descent, we found no association in French-Canadian AIS patients. This second replication cohort suggests that the lack of association of these SNPs in a Japanese cohort is not due to ethnicity.

Whole-exome Sequencing (WES) Data – Discovery phase

From this preliminary discovery phase, severe cases are enriched in rare variants (MAF < 0.01 from 1000 genomes and ESP databases) for 124 genes ($p < 0.05$). Next, a search for very rare single coding variants (MAF < 0.005 from databases), significantly associated with severe cases ($p < 0.05$), returned 29 very rare SNVs, thus highlighting 29 additional candidates.

A similar approach was performed with exome data for 13 severe AIS and 13 control samples from an Italian cohort, and additional rare DNA variants were found.

Suggestive results will be validated in future studies that include a large confirmation cohort of French-Canadian patients (recruitment ongoing), as well as collaborations for meta-analyses.

Expression profiling data.

Validation of selected candidate genes was carried-out at the RNA level by qPCR and at the protein level by Western blots and ELISA methods. Among the 38,000 human genes tested, we have found genes specifically associated with each functional subgroup while a few other genes were shared and affected to the same extent in all AIS functional subgroups suggesting their involvement in the genetic predisposition to elaborate upon a specific curve pattern (manuscript in preparation).

Sources and references

1.Connor JM, Conner AN, Connor RA et al. Genetic aspects of early childhood scoliosis. Am.J.Med. Genet. 1987;27:419-424.

2. Machida M. Cause of idiopathic scoliosis. Spine 1999;24:2576-2583.

3. Miller NH. Cause and natural history of adolescent idiopathic scoliosis. Orthop. Clin. North Am 1999; 30:343-52, vii.

4. Miller NH. Genetics of familial idiopathic scoliosis. Clin.Orthop. 200260-64.

5. Miller NH, Schwab DL, Sponseller PD et al. Characterization of idiopathic scoliosis in a clinically well-defined population. Clin.Orthop. 2001349-357.

6. Wise CA, Barnes R, Gillum J et al. Localization of susceptibility to familial idiopathic scoliosis. Spine 2000;25:2372-2380

7. Gorman, KF, Julien, C; Oliazadeh, N; Tang, Qn; and Moreau, A. (January 2014) Genetics of Idiopathic Scoliosis. In: eLS. John Wiley & Sons, Ltd: Chichester. DOI: 10.1002/9780470015902.a0025313

8. Gorman KF, Julien C. and Moreau A. The genetic epidemiology of idiopathic scoliosis. Eur Spine J. 2012 Oct; 21(10):1905-19. doi: 10.1007/s00586-012-2389-6.

9. Sharma S, Gao X, Londono D, Devroy SE, Mauldin KN, Frankel JT, Brandon JM, Zhang D, Li QZ, Dobbs MB, Gurnett CA, Grant SF, Hakonarson H, Dormans JP, Herring JA, Gordon D, Wise CA. Genome-wide association studies of adolescent idiopathic scoliosis suggest candidate susceptibility genes. Hum.Mol.Genet. 2011.

10. Qiu XS, Lv F, Zhu ZZ, Qian BP, Wang B, Yu Y, Qiu Y. Lack of association between the chl1 gene and adolescent idiopathic scoliosis susceptibility in han chinese: A case-control study. BMC musculoskeletal disorders. 2014;15:38.

11. Takahashi Y, Kou I, Takahashi A, Johnson TA, Kono K, Kawakami N, Uno K, Ito M, Minami S, Yanagida H, Taneichi H, Tsuji T, Suzuki T, Sudo H, Kotani T, Watanabe K, Chiba K, Hosono N, Kamatani N, Tsunoda T, Toyama Y, Kubo M, Matsumoto M, Ikegawa S. A genome-wide association study identifies common variants near lbx1 associated with adolescent idiopathic scoliosis. Nature genetics. 2011;43:1237-1240.

12. Fan YH, Song YQ, Chan D, Takahashi Y, Ikegawa S, Matsumoto M, Kou I, Cheah KS, Sham P, Cheung KM, Luk KD. Snp rs11190870 near lbx1 is associated with adolescent idiopathic scoliosis in southern chinese. Journal of human genetics. 2012;57:244-246.

13. Gao W, Peng Y, Liang G, Liang A, Ye W, Zhang L, Sharma S, Su P, Huang D. Association between common variants near lbx1 and adolescent idiopathic scoliosis replicated in the chinese han population. PloS one. 2013;8:e53234.

14. Kou I, Takahashi Y, Johnson TA, Takahashi A, Guo L, Dai J, Qiu X, Sharma S, Takimoto A, Ogura Y, Jiang H, Yan H, Kono K, Kawakami N, Uno K, Ito M, Minami S, Yanagida H, Taneichi H, Hosono N, Tsuji T, Suzuki T, Sudo H, Kotani T, Yonezawa I, Londono D, Gordon D, Herring JA, Watanabe K, Chiba K, Kamatani N, Jiang Q, Hiraki Y, Kubo M, Toyama Y, Tsunoda T, Wise CA, Qiu Y, Shukunami C, Matsumoto M, Ikegawa S. Genetic variants in gpr126 are associated with adolescent idiopathic scoliosis. Nature genetics. 2013;45:676-679.

Publications

1. Letellier K, Azeddine B, Parent S, Labelle H, Moreau A, and Moldovan F. Estrogens Crosstalk with the Melatonin Signalling Pathway in Human Osteoblasts derived from Adolescent Idiopathic Scoliosis (AIS) patients. J. Pineal Res. 2008, 45(4): 296-300 [Epub 2008 May 26].

2. Gorman KF, Julien C. and Moreau A. The genetic epidemiology of idiopathic scoliosis. Eur Spine J. 2012 Oct; 21(10):1905-19. doi: 10.1007/s00586-012-2389-6.

3. Gorman, KF, Julien, C; Oliazadeh, N; Tang, Qn; and Moreau, A. (January 2014) Genetics of Idiopathic Scoliosis. In: eLS. John Wiley & Sons, Ltd: Chichester. DOI: 10.1002/9780470015902.a0025313

4. Yadav MC, Huesa C, Narisawa S, Hoylaerts MF, Moreau A, Farquharson C, Millán JL. Ablation of osteopontin improves the skeletal phenotype of phospho1(-/-) mice. J Bone Miner Res. 2014 Nov; 29(11):2369-81. doi: 10.1002/jbmr.2281. PMID: 24825455

5. Tang QL, Julien C, Eveleigh R, Bourque G, Franco A, Labelle H, Grimard G, Parent S, Ouellet J, Mac-Thiong JM, Gorman KF, Moreau A. A Replication Study for Association of 53 Single Nucleotide Polymorphisms in ScoliScoreTM Test with Adolescent Idiopathic Scoliosis in French-Canadian Population. Spine (Phila Pa 1976). 2015 Feb 2. [Epub ahead of print] PMID: 25646748

CHAPTER 5

Screening for progressive scoliosis

One of the main goals of the research in idiopathic scoliosis is the detection and early diagnosis of the evolutive forms.
The objectives underlying theses project aim at achieving an early treatment of the evolutive deformities, avoiding the treatment of the non evolutive forms of scoliosis and maybe in the future, preventing the disease.
Vestibular tests, imagery or blood tests have been developed to understand the disease at its early stage.

A severity index for early detection of progressive scoliosis

Wafa Skalli and her team from Arts et Métiers ParisTech have a strong background in 3D geometric and mechanical modelling of the spine, and participated in the design of the EOS system, an innovative low dose bi-planar X-Ray system allowing for 3D reconstruction. Support was provided by the Cotrel Foundation for a research program on key biomechanical factors in relation with scoliosis progression. This was the topic of 3 PhDs, 2 of them being engineers (N. Champain, X. Drevelle), and one orthopaedic surgeon (A. Courvoisier). Multicentric data collection was initiated with the teams of Dr Ebermeyer and Courtois in Saint Etienne, and extended further to Lyon hospital and to several other centers, with an active involvement of C. Vergari, Post-Doctoral researcher,

The etiology of idiopathic scoliosis is multifactorial and unclear, and early detection of progressive curves is still a challenge. Scoliosis is associated with three dimensional global and local changes of the spine including lateral and axial deviations and rotations. In the growing spine these changes yield local hyper-pressure that can affect bone growth and disc material properties [1], which can in turn alter behaviour and induce curve evolution. Also muscle forces can become asymmetric for posture regulation. Such a vicious circle (also called biomechanical cascade) is not yet fully understood: we still do not know the key factors which explain the progression of a given mild scoliosis curve while another one, similar in appearance, remains stable with time. Currently used predictive factors include gender, remaining skeletal growth, curve location and magnitude [2]. Most of the follow up is based on frontal X-Rays, which only provide a projection of the 3D curve. Treatment decision is only taken once progression occurs, while early detection would be essential for treatment efficiency.

The hypothesis was that 3D subject specific biomechanical modelling should provide further understanding of the biomechanical cascade, and that thorough quantitative 3D analysis scoliotic spines, together with the use of data mining, could be useful for early detection of progressive curves.

As for biomechanical modelling, a previously validated subject specific biomechanical model was used for the spine of 18 adolescents, 12 with mild thoracolumbar scoliosis and 6 asymptomatic [3]. Accurate 3D reconstructions were the basis of each model, then a large set of conditions were simulated in order to induce curve progression in relation with gravity loads, disc stiffness and postero-anterior asymmetric vertebral growth. For some of the mild scoliosis curves, numerical simulation was able to mimic progression to severe scoliosis, with large rotations and torsion, thus providing possible explanations for biomechanical cascade. Surprisingly, simulations never resulted in such scoliosis like deformity for asymptomatic spines, clearly indicating that this biomechanical cascade is a secondary factor that can take place only once a specific deformation pattern is already there.

Thus efforts were made on thorough observation of scoliosis curves and to search for specific patterns that could early mark progressive spines. Large datasets of 3D reconstructions were collected for asymptomatic spines, spines with severe scoliosis requiring orthopaedic or surgical treatments, and mild scoliosis. A specific deformation pattern was first identified for severe scoliosis, particularly in relation with deformities in the transverse plane, and it appeared that seeds of such pattern could be

found at a very early stage [4].A specific mathematical method (Factorial Discriminant Analysis) was then used to assess, for each mild scoliotic curve, its «similarity» either to severe or to asymptomatic curves, thus yielding the definition of a severity index. The validation of this Severity index required a long follow up from the first exam when it was calculated to the end or growth or brace decision in order to blindly assess its relevance.

Results

Follow up is still in progress, but on the first 56 patients that were blindly followed until progression status was known, the Severity index set at the first exam was in agreement with the real final outcome in more than 80% of the cases [5], showing its great potential.

Figure 1 shows two very mild scolioses (10° Cobb angle), and for one of them the top view clearly shows the initiation of the torsion responsible for the scoliosis progression. This research also allowed stressing on the role of the intervertebral discs, and further basic research is now also focused on in vivo assessment of intervertebral discs [6]. In parallel, research is ongoing on brace simulation using subject specific models in order to improve brace efficiency. All these approaches are complementary and progressively converge to assess possible biomechanical pathomechanisms and key factors that could help for early detection of progression risk in mild scoliosis and for the assessment of strategies for improving treatment.

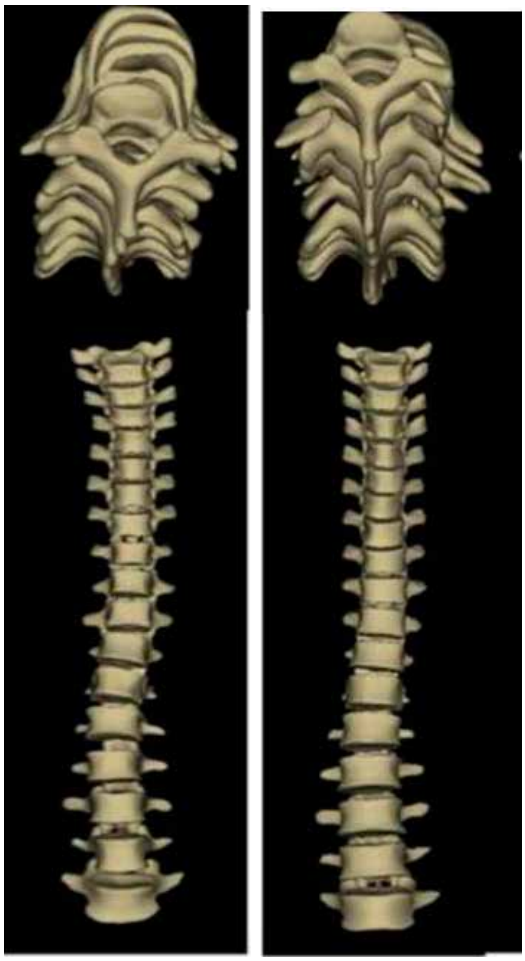


Fig 1, Courvoisier et al. Eur Spine J. 2013

References

1. Meir AR, Fairbank JC, Jones DA, McNally DS, Urban JP. High pressures and asymmetrical stresses in the scoliotic disc in the absence of muscle loading. Scoliosis. 2007 Feb 24;2:4.

2. Duval-Beaupère G, Lamireau T. Scoliosis at less than 30 degrees. Properties of the evolutivity (risk of progression). Spine. 1985 Jun;10(5):421-4.

3. Drevelle X, Lafon Y, Ebermeyer E, Courtois I, Dubousset J, Skalli W. Analysis of idiopathic scoliosis progression by using numerical simulation. Spine 2010 May 1;35(10):E407-12.

4. Courvoisier A, Drevelle X, Dubousset J, Skalli W. Transverse plane 3D analysis of mild scoliosis. Eur Spine J. 2013 Nov;22(11):2427-32.

5. Vergari C, Abelin-Genevois K, Kohler R, Drevelle X, Ebermeyer E, Courtois I, Dubousset J, Skalli W. A preliminary validation of a severity index for early detection of progressive adolescent idiopathic Scoliosis. Scoliosis Research Society 49th anual meeting, 2014, Anchorage

6. Vergari C, Dubois G, Vialle R, Gennisson JL, Tanter M, Dubousset J, Rouch P, Skalli W. Lumbar annulus fibrosus biomechanical characterization in children by ultrasound shear wave elastography, in revisión in Europ Radiology

7. Vergari C, Ribes G, Aubert B, Adam C, Miladi L, Ilharreborde B, Abelin-Genevois K, Rouch P, Skalli W. Evaluation of a patient-specific finite element model to simulate conservative treatment in adolescent idiopathic scoliosis, Accepted in Spine Deformity Journal

Predictive factor of progression in adolescent idiopathic scoliosis (AIS) - a multicentric study

Robert Debré hospital associates in this project a productive orthopedic department with Keyvan Mazda & Brice Ilharreborde and a Balance Functional Exploration center specialized in children (EFEE) with an international reputation (**Sylvette R Wiener-Vacher** & Maria Pia Bucci).

Research projects on AIS proposed before by this team were granted by the Fondation Cotrel (Vestibular otolith asymmetries in AIS published in 1998

and predictive value of Vestibular otolith asymmetries for potential progression of AIS: 2004)

This multicentric project is ambitious (collaboration between orthopedics and ORL department in 3 centers Paris, Lyon & Nancy). It will combine different approaches on predictive factors for AIS progression already identified by other teams granted by the Fondation Cotrel (biochemical and genetic approach with A. Moreau from Montreal, radiological approach with W. Skalli W ENSAM Paris and EOS, neurosensorial approach with posturographic index of AIS severity (Haumont et al 2011) and oculomotor index of AIS severity (Lion et al , 2014). This project will validate the predictive power of all parameters on the French population of AIS. The similar expertise of the 3 centers involved will permit the recrutment of a large AIS population with a well defined phenotyping (using identical methodology that is EOS 3D spine reconstruction). This appears to be the only way to determine wich of these factors or which combination of these factors can accurately predict and distinguish at onset the AIS that will be progressive and need urgent treatment to prevent further deformity (early bracing) from the AIS with no progression potential that only need observation.

Background :

Our team was interested initially in neurosensorial (postural and occulomotor) abnormalities that could be expressed and characterize AIS. Then the first results obtained on small populations suggested that these abnormalities depended on the severity of the AIS. We observed in some followed up cases that changes over time of these neurosensorial parameters could be correlated to the progression of spine deformity and could be used as predictive factors for AIS progression.

Neurosensorial posturographic factors:

Posturographc recording in various experimental conditions shows in progressive AIS less mastery of balance control in situations requiring proprioceptive information, or requiring vestibular information, or requiring vestibulo-proprioceptive feedback (Haumont et al, 2011). Progressive AIS versus non progressive AIS shows higher body sway in static tests with eyes open, characterized by a higher sway path of the center of foot pressure and higher anteroposterior oscillations. They have poorer balance than controls, in the standard conditions (eyes open, stable visual surrounding and stable platform), when only somatosenrory cues are disrupted (eyes open, stable visual surround but sway-referenced platform), and when visual and somatosenrory cues are disrupted (eyes open but sway-referenced visual surrounding and sway-referenced platform).

Neurosensorial oculomotor factors:

AIS with more severe Cobb angle (15°-25°) at onset compared to milder Cobb angle (<15°) presented abnormal oculo-vestibular coupling in absence of any vestibular or ocular pathology and abnormal voluntary and réflex saccades (longer latencies and smaller velocities) in some demanding conditions (Lion et al 2013)

Asymetries of the otolithic vestibulo-ocular responses were also observed in AIS patients and appeared in

few asymptomatic cases prior to the onset of their scoliosis and increasing During the rapid growth period in progressive AIS suggesting that these asymmetries from central origin may preceed the spine deformities and could predict AIS (Wiener-Vacher & Mazda, 1998).

Hypothesis and Methodology:

What are we looking for? Validate in the French pediatric population the contribution of specific markers: biological endophenotypes (osteopontin and soluble sCD44 receptor blood levels, and evaluation of Gi-coupled receptor signaling), radiological phenotypes and neurosensorial markers that can predict the risk of AIS progression at the earliest stage in symptomatic children and distinguísh severe progressive scoliosis that needs urgent treatment (bracing and/or surgery) from non progressive AIS that needs only observation.

Which methodology?

- Spine biomechanical properties will use the 3D spine reconstruction EOS system to precisely determine the phenotype and calculate a severity index.
- Neuro sensorial evaluation including posturographic characteristic, vestibulo-ocular reponses, optocinetic ocular responses, voluntary and reflex saccade parameters will detect abnormalities found only in severe AIS.
- Determination of biological endophenotypes using osteopontin plasma levels, soluble CD44 receptor blood levels, and G-protein-coupled receptors (Gi) dysfunction measured by peripheral blood cell reactivity to the idiomelatonin (CDS method). This will give 3 distinct groups of AIS patients with high risk of progression, medium risk of progression and low risk of progression.

Results:

This project is an ongoing 4 years project proposed for a National PHRC (2015)

Publications on previous projects:

Wiener Vacher Sylvette & Mazda Keyvan. Asymmetric otolith vestibulo-ocular responses in children with idiopathic scoliosis. J Pediatr. 1998 Jun;132(6):1028-32.PMID: 9627598

Lion A, Haumont T, Gauchard GC, Wiener-Vacher SR, Lascombes P, Perrin PP. Visuo-oculomotor deficiency at early-stage idiopathic scoliosis in adolescent girls. Spine (Phila Pa 1976). 2013 Feb 1;38(3):238-44. doi: 10.1097/BRS.0b013e31826a3b05.PMID:22828711

Ilharreborde B, Dubousset J, Le Huec JC. Use of EOS imaging for the assessment of scoliosis deformities: application to postoperative 3D quantitative analysis of the trunk. Eur Spine J. 2014 Jul;23 Suppl 4:S397-405. doi: 10.1007/s00586-014-3334-7. Epub 2014 May 9. Erratum in: Eur Spine J. 2014 Jul;23 Suppl 4:S468. PMID: 24811688

CHAPTER 6

Perspectives

The following chapter gathers the new projects supported this year by the Fondation Cotrel. They are still at a preliminary stage but their potential is high and cover new aspects which have been less studied until now.

Role of microRNAs in Adolescent Idiopathic Scoliosis Etiology A Canada-China-Italy Collaborative

Dr. Alain Moreau,
Dr. Stefan Parent (MD, PhD, Montreal, Canada) is a clinical-scientist and orthopaedic spine surgeon who has beencollaborating with Dr. Moreau since 2001. He will assist Dr. Moreau in all clinical aspects related to spinal deformity progression and he will coordinate the clinical evaluation of the French-Canadian cohort.
Dr. Jack. C. Cheng (MD, Hong Kong, China)
Dr. Marco Brayda-Bruno (MD, PhD, Milan, Italy) is a leading spine surgeon and clinical-scientist who has been collaborating with Dr. Moreau since 2008. He will assist Dr. Moreau in the clinical validation of selected miRNAs using his Italian AIS cohort (Milan, Italy).

This new research project has just started 10 months ago and it aims to identify small fragments of RNA called microRNAs that are circulating in the blood of patients with adolescent idiopathic scoliosis (AIS). In addition to their role as potential biomarkers, circulating microRNAs are also involved in many diseases because they can block the activity of several genes (up to 200 genes), which explains our interest to study them in the context of AIS. We recently identified several microRNAs in plasma from AIS patients associated with one of three functional groups initially identified in the project 1 funded by The Yves Cotrel Foundation, which appear to be associated with severe scoliosis. MicroRNAs selected as part of our study will be validated in different populations (Canadian-French, Chinese and Italian) on a larger scale and longer term will enable the development of diagnostic and prognostic tools to help clinicians identify asymptomatic children's risk of developing scoliosis and to offer new therapeutic approaches for those affected.

Background

Adolescent Idiopathic Scoliosis (AIS) is a common complex genetic disease and one of the most prevalent childhood deformities.[1,2] It presents both an immediate medical challenge and a chronic condition that affects individuals throughout their lives. On average AIS affects 4% of the global pediatric population.[3] Although the syndrome is phenotypically complex with a recognized genetic component, it is clinically characterized by a 3D spinal deformity of unknown etiology.[4] Most patients requiring corrective surgery are females (90%), and the cause of this gender bias remains unexplained.[3] Although advancements in genomic technologies are transforming the genetic landscape and research field, there is so far only limited success in deciphering the mechanisms of complex diseases such as AIS, thereby allowing the translation of these discoveries to clinical applications in order to improve health and reduce the socio-economical burdens associated with current treatments. Therefore, it is mandatory to explore the possible contribution of microRNAs (miRNAs) in the AIS etiopathogenesis as miRNAs have recently been shown to play a key role in numerous pathophysiological processes, which will provide new opportunities for identifying both functional drivers and specific biomarkers for AIS.
MicroRNAs (miRNAs) are a class of small noncoding RNAs that are involved in a range of physiological processes, with each miRNA regulating on average 200 target genes. Many genes contain target sites for one or more miRNAs. Different forms of miRNAs, which target specific mRNAs, can regulate gene expression in a tissue-specific manner.[5] Circulating miRNAs present in human plasma or serum have become an emerging field of study in biomedical research, mostly due to their potential applications in the diagnosis and prognosis of several diseases.
Recently, growing evidence has shown that miRNAs are taken in by intracellular exosomes, secreted into circulation, and taken up by other cells. The circulating

levels of several miRNAs have been shown to be altered in diseases such as cancer, diabetes, and cardiovascular diseases, and therefore are suggested to regulate critical functions of the recipient cells by modulating protein expression.

The stability of miRNAs outside the cells, combined with the ease of sample collection (blood) makes it easier to both detect and measure them and especially, to use them as non-invasive biomarkers.[6] Recently, circulating miRNAs have been proven to be involved in complex diseases like Duchenne muscular dystrophy (DMD), where scoliosis is a common complication[7]. In a recent study published in 2013, researchers identified miRNAs that could be used as serum biomarkers for monitoring DMD severity[8]. They found that certain miRNAs (e.g. miR-1, miR-133 a, b, miR-206) were up-regulated in the serum of DMD patients. Interestingly, DMD patients with severe scoliosis had significantly low levels of miR-1, miR-31 and miR-133 compared to those with mild or no scoliosis.

Despite the fact that we do not know *a priori* if these miRNAs are involved in scoliosis onset and/or spinal deformity progression in DMD and/or AIS patients, this paper prompted our interest for the following reasons:
1. Ikeda et al. (2009)[9] reported that calmodulin (CaM) expression is inhibited by miR-1, which could explain the up-regulation of CaM in AIS patients exhibiting severe spinal deformities as previously shown by the work of Lowe et al. In addition, treatment of scoliosis animal models with CaM pharmacological inhibitors led to a reduction in the severity of spinal deformities.[10,11]

2. Integrin subunits $\alpha 5$, $\alpha 1$ and $\beta 3$ are known targets of miR-31, which represses their expression.[12] We recently discovered that Gi-signalling impairment occurring in AIS is mediated by osteopontin (OPN) signalling activity predominantly through its interaction with $\alpha 5\beta 1$ integrins in AIS (Akoume et al., 2015, manuscript in revision).
Furthermore, changes in circulating and tissue miRNA levels in response to exercise[13] and the discovery of mechanosensitive microRNAs[14], suggest that some circulating miRNAs could be involved in the regulation of mechanotransduction and could be clinically relevant to explain differences in brace responsiveness among AIS patients.

Hypothesis

Certain miRNAs could be involved in AIS etiology. Indeed, misregulation of key genes by miRNAs may somewhat explain AIS pathogenesis. This hypothesis is further strengthened by our preliminary data showing an up-regulation of miR-224 (+2.4 fold) and down-regulation of miR-145 (-2.3 fold) and miR-624 (-4.3 fold) expression in osteoblasts derived from a subset of AIS patients.
This project will fill the current gaps in our knowledge of AIS etiology. To the best of our knowledge, the possible contribution of miRNAs in spinal deformity onset and/or progression has not been investigated so far. If proven, the use of circulating miRNAs as biomarkers could lead to an affordable low cost assay to predict not only scoliosis and/or the risk of disease progression but could differentiate clinical outcomes as well.
Improving patient stratification at an earlier stage, in combination with new clinical decision-making tools and algorithms, should also reduce the serious psychological effects of interventions, decrease the burden of scoliosis by avoiding unnecessary cumbersome treatments (e.g. brace) in patients who will not respond to the treatments due to their genetic predisposition, as well as reduce hospital visits and costly non-surgical and surgical interventions.

Methodology

Circulating miRNAs will be prepared from filtered platelet-poor plasma, which wll be then proceed to RNA isolation, using the miRNeasy kit (Qiagen), following manufacturer's instructions. Then, genome-wide miRNA expression profiling will be performed using the Agilent expression array-Human miRNA 8x60k. Selected circulating miRNA identified in our discovery set will first be validated by quantitative real-time PCR (qPCR) using the miRCURY LNA™ Universal RT microRNA PCR kit (Exiqon, MA, USA) on a 7900HT real time PCR system (Applied Biosystems) according to the manufacturer's protocol.

Results

This project was just begun in January 2015. Understanding that AIS is a common complex genetic disease, our initial microRNA inquiry led us to identify three miRNAs significantly associated with AIS patients classified in biological endophenotype FG1 through Affymetrix microarray analysis.

Indeed, we discovered that mir-224 was 2.4 times up-regulated in osteoblasts derived from AIS patients classified in FG1 ($p=0.04$) when compared to patients from the FG2 and FG3 subgroups. Interestingly, mir-145 and mir-622 were 0.43 and 0.23 times down-regulated in osteoblasts derived from FG1 patients ($p=0.006$ and $p=0.026$) when compared to FG2 and FG3 patients. We are in the process of identifying and validating the transcriptional targets of these three microRNAs. In parallel, we are investigating the possible contribution of circulating microRNAs in AIS pathogenesis.

Sources and references

1. Cheng JC, Tang NL, Yeung HY, Miller N. Genetic association of complex traits: using idiopathic scoliosis as an example. Clinical orthopaedics and related research 2007;462:38-44.

2. Wise CA, Gao X, Shoemaker S, Gordon D, Herring JA. Understanding genetic factors in idiopathic scoliosis, a complex disease of childhood. CurrGenomics 2008;9:51-9.

3. Brodner W, Krepler P, Nicolakis M, et al. Melatonin and adolescent idiopathic scoliosis. JBone Joint SurgBr 2000;82:399-403.

4. Society SR. Morbidity & Mortality Committee annual report; 1997.

5. Ameres SL, Zamore PD. Diversifying microRNA sequence and function. Nature reviews Molecular cell biology 2013;14:475-88.

6. Reid G, Kirschner MB, van Zandwijk N. Circulating microRNAs: Association with disease and potential use as biomarkers. Critical reviews in oncology/hematology 2011;80:193-208.

7. Wang CH, Bonnemann CG, Rutkowski A, et al. Consensus statement on standard of care for congenital muscular dystrophies. Journal of child neurology 2010;25:1559-81.

8. Zaharieva IT, Calissano M, Scoto M, et al. Dystromirs as serum biomarkers for monitoring the disease severity in Duchenne muscular Dystrophy. PloS one 2013;8:e80263.

9. Ikeda S, He A, Kong SW, et al. MicroRNA-1 negatively regulates expression of the hypertrophy-associated calmodulin and Mef2a genes. Molecular and cellular biology 2009;29:2193-204.

10. Akel I, Demirkiran G, Alanay A, Karahan S, Marcucio R, Acaroglu E. The effect of calmodulin antagonists on scoliosis: bipedal C57BL/6 mice model. European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society 2009;18:499-505.

11. Akel I, Kocak O, Bozkurt G, Alanay A, Marcucio R, Acaroglu E. The effect of calmodulin antagonists on experimental scoliosis: a pinealectomized chicken model. Spine (Phila Pa 1976) 2009;34:533-8.

12. Chen W, Harbeck MC, Zhang W, Jacobson JR. MicroRNA regulation of integrins. Translational research : the journal of laboratory and clinical medicine 2013;162:133-43.

13. Banzet S, Chennaoui M, Girard O, et al. Changes in circulating microRNAs levels with exercise modality. J Appl Physiol (1985) 2013;115:1237-44.

14. Marin T, Gongol B, Chen Z, et al. Mechanosensitive microRNAs-role in endothelial responses to shear stress and redox state. Free radical biology & medicine 2013;64:61-8.

High-resolution spine muscle MRI and whole Body muscle MRI, comparative study in idiopathic scoliosis and neuromuscular scoliosis

In Raymond Poincaré hospital a multidisciplinary team is in charge of the medical care of scoliosis either in idiopathic scoliosis or in deformities associated with inherited muscle disorders.
Dr Catherine Marty in charge of rehabilitation of idiopathic scoliosis, Pr Susana Quijano-Roy in charge of the local pediatric neuromuscular reference center, Dr Marie line Pissonnier a young spine surgeon and **Pr Robert-Yves Carlier** head of medical imaging department constitute the team.
Pr Carlier and Pr Quijano-Roy have a strong experience in muscle MRI as a clinical research tool in inherited muscle disorders especially in congenital myopathies and congenital muscular dystrophies.

Background

In congenital myopathies and congenital muscular dystrophies whole body muscle MRI not only shows muscle alterations in limbs but also in spine muscles.
Alterations (fatty infiltration, atrophy) of the muscles of the back are most of the time very similar in the same disease.
In the same manner the type of spine deformity is similar for the same disease.
However In some diseases even with alterations of spine muscles there is no spine deformity. On the contrary some diseases with only slight alterations in back muscles are associated with important deformities.

The goal of this work is to describe early modifications in spine muscles in idiopathic scoliosis of infants and adolescents, using high field and high resolution MRI (3T).
The goal is also to compare the MRI results with those of similar MRI studies in infants and adolescents with deformities linked to inherited muscular disorders.
Comparison of the type and degree of spine deformities has never been made with techniques associated with such spatial resolution.
Detection of localized denervation or other spine muscle dysfunction signs with diffusion has never been evaluated.

Methodology

High field MRI can explore in a reasonable time and with sufficient resolution all muscles around the spine.
High field MRI can explore in a reasonable time and with sufficient resolution all muscles around the spine.

Patients with inherited muscle disorders will be enrolled by the neuro-paediatric unit (Pr S. Quijano-Roy) and patients with idiopathic scoliosis by the scoliosis consultation (Dr C Marty) during a period of three years.

First step, the pilot study
Technical MRI parameters adaptations in five young adults volunteers.

Second step , enrollment of patients in three subgroups
- five patients with idiopathic scoliosis,
- five patients with scoliosis associated with inherited muscle disorders
- five volunteers

Third step, associated explorations and MRI post-processing
- Muscle Testing (axial-proximal protocol)
- Motor function scale MFM
- Pulmonary function tests
- EOS

(initially and every six months in case of deformity or more frequently adapted to speed of deformity progression ; 2D and 3D measurements).
- Whole body muscle MRI in all patients initially
Axial and frontal T1 w, axial STIR images

Qualitative description of muscle involvement based on fatty replacement and atrophy.

- Spine Muscle high-resolution each 6 months (GE 3T discovery 750 W)
3D HR T1 and T2w sequences.
Axial DWI
Axial IDEAL IQ(DIXON 3 points)
Post-processing in a dedicated work-station

Expected Results

- High resolution anatomic atlas of axial muscles
- Feasibility of spine muscle HR MRI in idiopathic scoliosis in child and adolescent
- Complementarity with EOS 3D modeling
- Better knowledge of muscles lesions associated with spine deformities
- Better identification of muscles mostly modified initially and during the natural history of idiopathic scoliosis
- Determination of common and differential modifications in axial muscles of idiopathic and non-idiopathic scoliosis
- Description of axial muscle modifications in different types of deformities
- Evaluation of axial muscle HR MRI as a tool in scoliosis care
- Obtain more precise anatomic details of axial muscle involvement in inherited muscles disorders.

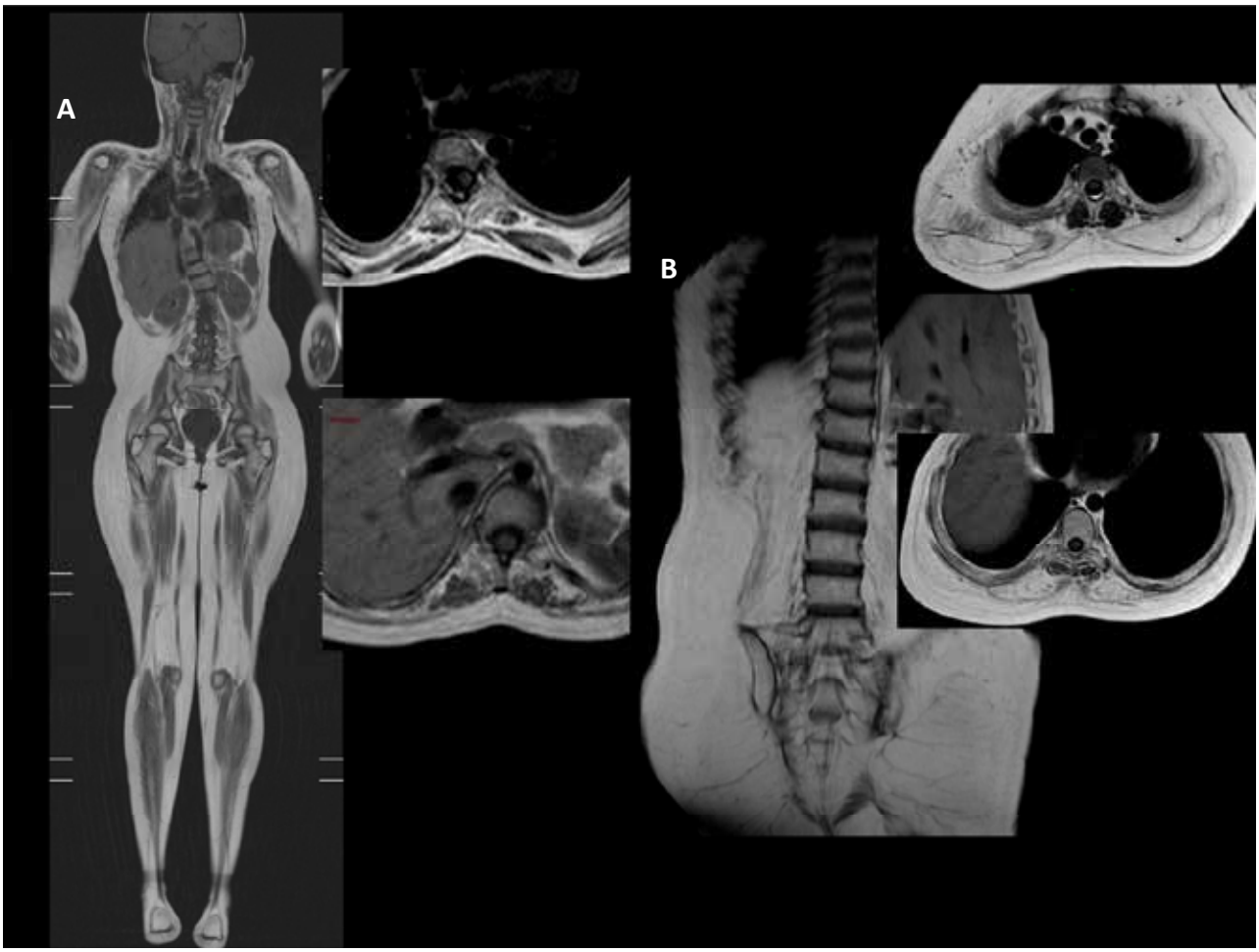


Illustration of WB MRI of two different inherited muscular disorders.
On the left (A) frontal and axial T1 w views of SEPN1 or rigid spine muscular dystrophy
On the right (B) frontal and axial T1 w views of a Duchenne muscular dystrophy
Spinal muscles are partly fatty involved in both diseases but the involvement has not the same distribution.
In SEPN 1 the scoliosis is important , in Duchenne MD there is structural scoliosis.

Publications

1. Quijano-Roy S1, Avila-Smirnow D, Carlier RY; WB-MRI muscle study group. Whole body muscle MRI protocol: pattern recognition in early onset NM disorders. Neuromuscul Disord. 2012 Oct 1;22 Suppl 2:S68-84.
2. Hankiewicz K, Carlier RY, Lazaro L, Linzoain J, Barnerias C, Gómez-Andrés D, Avila-Smirnow D, Ferreiro A, Estournet B, Guicheney P, Germain DP, Richard P, Bulacio S, Mompoint D, Quijano-Roy S. Whole body muscle MRI in SEPN1-related myopathy shows a homogeneous and recognizable pattern. Muscle Nerve. 2015 Mar 21. [Epub ahead of print]
3. Jarraya M, Quijano-Roy S, Monnier N, Béhin A, Avila-Smirnov D, Romero NB, Allamand V, Richard P, Barois A, May A, Estournet B, Mercuri E, Carlier PG, Carlier RY. Whole-Body muscle MRI in a series of patients with congenital myopathy related to TPM2 gene mutations.Neuromuscul Disord. 2012 Oct 1;22 Suppl 2:S137-47.
4. Carlier PG, Azzabou N, de Sousa PL, Hicks A, Boisserie JM, Amadon A, Carlier RY, Wary C, Orlikowski D, Laforêt P. Skeletal muscle quantitative nuclear magnetic resonance imaging follow-up of adult Pompe patients. J Inherit Metab Dis. 2015 Mar 7. [Epub ahead of print]
5. Carlier RY, Laforet P, Wary C, Mompoint D, Laloui K, Pellegrini N, Annane D, Carlier PG, Orlikowski D. Whole-body muscle MRI in 20 patients suffering from late onset Pompe disease: Involvement patterns. Neuromuscul Disord. 2011 Nov; 21(11): 791-9.

The role of gut hormones in adolescent idiopathic scoliosis pathophysiology: a pilot study

This project was initiated and performed at Sainte-Justine Hospital, in **Dr Valérie Marcil**'s laboratory. Dr Marcil is a professor researcher at the Department of Nutrition, Faculty of Medicine – Université de Montréal, Researcher at the Sainte-Justine University Health Center.
The research team of this project is composed of a network of collaborators specialized in AIS in Montreal (Drs Moreau and Moldovan), in Quebec city (Dr Simoneau) and in Italy (Drs Colombini and Brayda-Bruno) and in nutrigenomics (Dr Levy), genetics, microbiota and data analysis (Drs Amre and Barreiro).

Background

Several studies have found that patients with adolescent idiopathic scoliosis (AIS), especially girls, have common features of taller stature, lower body mass index (BMI) and systemic low bone mass[1-5], but the causes of these anthropometrical differences mostly remain unexplained. In this research project, we propose that several factors participating in regulating energy expenditure and metabolism could contribute to explain anthropometrical features and perhaps disease development and/or progression. Many hypotheses have been proposed to explain the alleged association between abnormal growth pattern and AIS. Among them, leptin, a hormone primarily produced by the adipose tissue, has been a candidate for the etiology of AIS. Some studies have reported lower serum leptin levels in girls with AIS that correlated with weight, BMI and body mineral density[6], as well as with trabecular bone parameters[7], but others did not find any differences[8]. Levels of leptin and adiponectin at 10 years old was found predictive of scoliosis development at age 15[9] but variations in the *leptin* gene were not found associated with AIS in Chinese[10] or in Hungarian patients[11]. It was proposed that abnormal leptin bioavailability[8] and low leptin expression in bone mesenchymal stem cells and osteoblasts[10] could be involved in the etiopathogenesis of AIS. Although highly interesting, the role of leptin in AIS etiopathology has not yet been proven conclusive.

From a metabolic perspective, energy homeostasis (such as appetite regulation, insulin sensitivity, bone mass and bone growth) is maintained by the interplay between several hormonal regulators that are not only secreted by the adipose tissue (adipokines), but also by the gastrointestinal (GI) tract in response to nutrient intake. In addition to its roles in regulating energy homeostasis, appetite[12, 13] and glucose metabolism[14], the gut is emerging as a fundamental regulator of bone health. The GI-secreting peptides [namely glucagon like peptide (GLP)-1, GLP-2, glucose-dependent insulinotropic polypeptide (GIP), ghrelin and peptide YY (PYY)] are incretins secreted after meal ingestion. They modulate glucose homeostasis, mainly through glucose-induced enhancement of insulin secretion and inhibition of glucagon release. For instance, it is of common knowledge that bone resorption decreases in the postprandial state (after a meal), leading to the suggestion that GI hormones may influence bone regulation[15].

Of particular interest, GLP-1, produced by intestinal endocrine L cells in response to nutrient intake, is a pluripotent incretin hormone in humans. Many beneficial extraglycemic actions of GLP-1 have been reported, specifically on body weight, blood pressure, dyslipidemia, cardiac and endothelial function[16]. GLP-1 action is tightly controlled by dipeptidyl peptidase-4 (DPP-4) as more than 50% of GLP-1 is inactivated before it reaches the systemic circulation[16]. GLP-1 binds to its receptor GLP1R and this interaction results in activation of intracellular signaling[17, 18].

Insulin metabolism is closely linked to the activity of bone cells as it binds to its specific receptor on osteoblasts (the insulin receptor, INSR) and stimulates the expression of osteocalcin, a hormone secreted by osteoblasts. In turns, osteocalcin

stimulates pancreatic ß-cell proliferation and insulin production[19,20]. It was demonstrated that osteocalcin is involved in glucose metabolism by increasing insulin secretion and cell proliferation in pancreatic â-cells and by upregulating the expression of the adiponectin gene in adipocytes, thus improving insulin sensitivity[21,22]. In adults, circulating osteocalcin levels are associated with improved glucose tolerance and insulin secretion and sensitivity[23,24].

Because insulin is known for its action in promoting bone growth[25], we believe that this phenomenon could contribute to scoliosis etiology or progression. Our preliminary data show that:

(1) Plasma DPP-4 is less abundant in AIS patients than in controls;
(2) DPP4 gene expression is lower in osteoblasts obtained from AIS patients compared to controls;
(3) The prevalence of a coding mutation in the GLP-1 receptor gene (*GLP1R*) is significantly higher is a AIS versus a control cohort;
(4) Plasma levels of osteocalcin are higher in AIS compared to control subjects.

Emerging evidence suggests that enteroendocrine peptides, such as GLP-1, are involved in the regulation of energy balance and glucose homeostasis via the digestion of specific dietary fibers or non-digestible carbohydrates by gut microbiota fermentation[26]. Data support a role for the microbiota in improving bone health: the ingestion of prebiotics such as inulin and fructo-oligosaccharides might help prevent osteoporosis[27,28]; and, in rats, prebiotic fibers increas bone calcium levels[29] and ameliorate the osteoporosis induced by ovariectomy by inhibiting osteoclast formation[30,31]. It is realistic to envisage that, by modifying microbiota composition, one could eventually modulate incretin secretion and, according to our hypothesis, benefit high-risk scoliotic patients.

Epigenetic, proteomic and functional studies of rare variants in AIS genes

The research project will be conducted by **Dr Moldovan**'s team, in collaboration with Dr Child and Aragon from. UK, (St. George's, University of London), **Dr Patten** from Montreal and several other collaborators from France (Dr Edery,, Dr Rousié) and from Canada (Dr Parent, Dr Simoneau, Dr Fortin and Dr Schmittbuhl).

This project is a logical extension of the work that identified POC5 gene as a gene responsible for familial AIS in French Families (1), and the work directed by Dr. Child from St. George's, University of London that identified Ocaka et al 2018 (2). The Researchers of this project are members International Consortium of Genetic of scoliosis. Dr Patten is a young researcher having considerable experience with the zebrafish animal model that in fact was the postdoctoral student in Dr Moldovan's laboratory at CHU Sainte Justine that discovered the POC5 gene variants as causative of familiar form of scoliosis. He is now, Professor at the INRS-Institut Armand-Frappier, and he is continuing to develop the in vivo animal models of scoliosis using the zebrafish approaches. Through a collaborative effort initiated in 2014 between the team of CHU Sainte Justine and Dr Child from St. George's, University of London, this project will investigate by Targeted Next Generation Sequencing of coding region of two genes using the AmpliSeq technology and through the epigenetic, proteomic and functional studies of rare variants in AIS genes.

Background

Explaining the historical context of the project.

This research proposal is a logical extension of our previous work on *poc5* gene. The overall goal is to identify consequences caused by gene variants responsible for adolescent idiopathic scoliosis (AIS), at the molecular and cellular levels (from gene variant to the defective protein) and to clarify how these mechanisms are triggered by pubertal hormone estradiol (E2).

This research proposal is a logical extension of our previous work on *poc5* gene. The overall goal is to identify consequences caused by gene variants responsible for adolescent idiopathic scoliosis (AIS), at the molecular and cellular levels (from gene variant to the defective protein) and to clarify how these mechanisms are triggered by pubertal hormone estradiol (E2).

Rationale

In the recent decades, genetic aspects of scoliosis have moved from relative obscurity, to a position of importance for understanding this disorder. Genetic analyses have identified several candidate loci predisposing IS, genes associated with AIS, gene variants involved in Congenital Scoliosis (CS) and finally, some rare genes variants that contribute to the occurrence of AIS (3-10) Since several years, a major causative gene of AIS is sought, but not identified. In contrast to the rare monogenic pathologies, most probably, AIS is not caused

by rare mutation in a single gene. As a complex multifactorial disorder (3, 11-14), with variable expressivity and penetrance, AIS definitely involves more than one gene, and perhaps those genes interact and/or share similar biological functions. Based on the recent data from our group and others (1, 2, 3, 4-10), **several of these genes play roles with the centrosome function, cell polarity, axons guidance, primary cilia and left-right axis determination.** In addition to rare variants in the intriguing centriolar *poc5* gene that we recently found in AIS Families of French ancestry, Dr Child identified another rare functional variants in a gene coding for a protein with very similar function (in AIS families from UK). Importantly the two genes appear to be functionally connected and play a role in centrosomal and/or extra-centrosomal microtubules (cilia organization). Thus, interesting hypothesis of a specific role of cilia in AIS etiopathogenesis could be proposed.

Our hypothesis is that sensory reception of cilia are affected by rare variants in genes producing defective proteins of primary cilia and centrosome.

Centrosomes are the principal microtubule-organising centres in somatic cells. They control cell shape, polarity, and the mitotic spindle. Mitotic centrosomes consist of two centrioles, cylindrical structures composed of microtubules, within the pericentriolar material. Centrioles also bind to the plasma membrane and act as the basal bodies for cilia. **Cilia are microtubule-based protrusions from the plasma membrane, that carry out motility and sensory functions.** Little is known about many of the proteins that regulate their structure and function. Intriguingly, the two genes (variants identified independently, in French and UK AIS patients) are ciliated proteins and share function and localization at the cilium basal body. For basal body assembly and function, centrins (small calcium-binding proteins related to the calcium-binding protein, **calmodulin) appears to play a central role.** Centrins assume a key role in ciliogenesis (rather than in centriole assembly). Humans have 4 centrins, including the centrin2, which directly co-localize and interact with POC5. In humans POC5 localize to the distal lumen of centrioles, where it is required for centriole elongation, and when overexpressed, POC5 induces formation of some elongated linear structures that look like primary cilia. Both primary and motile cilia exhibit sensory functions most of which are mechanosensory or chemosensory in nature. In addition to the mechanoreceptive properties, their sensory functions are exhibited by the TRP family receptors and Ca2+ influxes and interestingly, cilia responds to the sex hormons.

The effect of estradiol treatments on the structure and disposition of the centriolar complex was previously analyzed, but still fragmentary. It was found that E2 reduced the formation of the solitary (primary) cilia and affect the configuration of the paired centrioles. After E2 treatment, the number of cells that are baring cilia in the luminal epithelium, was found to be decreased, and both parent and daughter centrioles lacked basal feet, transitional fibers and rootlets. Several authors observed a transitory production of solitary (9+0) cilia preceded the appearance of motile (9+2) cilia in diverse epithelial cells which in fully differentiated state carry ciliated border.

Defects in both motile and primary cilia have been associated with a large number of human diseases, known as ciliopathy (genetic disorders of the cellular cilia or the cilia anchoring structures, the basal bodies, or of ciliar function) and abnormal musculoskeletal phenotype, including spinal deformity, is not infrequent in these disorders.

Our preliminary histological evaluation of POC5 mutants reveals some non-expected findings; lack of structural features of eye layer (inner nuclear layer; inner plexiform layer; and pigmented epithelium are affected), abnormal number of otoliths of ear and asymmetry of the midbrain, (where the mutated protein was principally but not exclusively accumulated. Of course, there we are missing data regarding the role POC5 in vivo. In human cells, POC5 localizes to the distal portion of centrioles and is recruited to procentrioles for full centriolar maturation and normal cell-cycle processing (15). This centrosomal protein interacts with centrin (16, 17) and inversin, both involved in cell division, polarity, and motility. Thus, the function of primary cilia and left-right axis determination may be somehow impaired in patients and connected to IS.

This is a unique opportunity to elucidate the molecular/mechanistic basis of AIS. This opportunity lies in the fact that rare variants were identified in two genes producing proteins that perform similar biological function (the first one in French families descents and the second one in UK AIS families)

The purpose of this proposal is to screen UK and French Canadian AIS patients for the

two mutated genes (causative of AIS) and to assess the shared pathogenic nature of both mutations.

To test our hypothesis that defect of cilia, centrosome and microtubule organization, are ultimately able to affect underlying cellular mechanisms responsible for AIS and E2 ability as triggering factor, the following research plan is proposed

Hypothesis and Methodology

The purpose of this proposal is to screen UK and French Canadian AIS patients for the two mutated genes (causative of AIS) and to assess the shared pathogenic nature of both mutations. To test our hypothesis that defect of cilia, centrosome and microtubule organization, are ultimately able to affect underlying cellular mechanisms responsible for AIS and E2 ability as triggering factor, the following research plan is proposed:

Screening UK and Canadian AIS patients for rare gene variants.To determine the prevalence of the causative mutation in a cohort of AIS families form Quebec and UK and sporadic cases, the presence or absence of the causative mutations will be determined using Ion AmpliSeq sequencing. Ion AmpliSeq™ technology brings simple and fast library construction for affordable targeted sequencing of specific genes or genomic regions, with low amount of DNA. At present, we developed such test and designed gene coverage with target on the exomes of the two gens. This test will be put into operation, to estimate gene variants prevalence in UK and Quebec population. DNA of 200 AIS patients (Families and cases, will be analysed, with careful attention provided to ethnicity, which is an important parameter in genetic study of mutation prevalence.

Publications

1. Patten SA, Margaritte-Jeannin P, Bernard JC, Alix E, Labalme A, Besson A, Girard SL, Fendri K, Fraisse N, Biot B, Poizat C, Campan-Fournier A, Abelin-Genevois K, Cunin V, Zaouter C, Liao M, Lamy R, Lesca G, Menassa R, Marcaillou C, Letexier M, Sanlaville D, Berard J, Rouleau GA, Clerget-Darpoux F, Drapeau P, **Moldovan F**, Edery P. Functional variants of POC5 identified in patients with idiopathic scoliosis. *J Clin Invest.* 2015 Mar 2;125(3):1124-8.
2. Ocaka L., Zhao C., Reed JA, Ebenezer ND, Brice G., Morley T., Mehta M., O'Dowd J., Weber JL., Hardcastle AJ, Child AH. Assignment of two loci for autosomal dominant adolescent idiopathic scoliosis to chromosomes 9q31.2-q34.2 and 17q25.3-qtel. J Med Genet 2008; 45:87-92.
3. Edery P, Margaritte-Jeannin P, Biot B, Labalme A, Bernard JC, Chastang J, Kassai B, Plays MH, Moldovan F, Clerget-Darpoux F. New disease gene location and high genetic heterogeneity in Idiopathic Scoliosis. *Eur J Hum Genet*. 2011 Aug; 19(8):865-9.
4. Kesling KL, Reinker KA. Scoliosis in twins. A meta-analysis of the literature and report of six cases. Spine (Phila Pa 1976) 1997;22:2009-14;
5. Axenovich TI, Zaidman AM, Zorkoltseva IV, Tregubova IL, Borodin PM. Segregation analysis of idiopathic scoliosis: demonstration of a major gene effect. American journal of medical genetics 1999;86:389-94
6. Wise CA, Barnes R, Gillum J, Herring JA, Bowcock AM, Lovett M. Localization of susceptibility to familial idiopathic scoliosis. Spine (Phila Pa 1976) 2000;25:2372-80
7. Bashiardes S, Veile R, Allen M, et al. SNTG1, the gene encoding gamma1-syntrophin: a candidate gene for idiopathic scoliosis. Human genetics 2004;115:81-9.
8. Sharma S, Gao X, Londono D, et al. Genome-wide association studies of adolescent idiopathic scoliosis suggest candidate susceptibility genes. Human molecular genetics 2011;20:1456-66.
9. Buchan JG, Gray RS, Gansner JM, et al. Kinesin family member 6 (kif6) is necessary for spine development in zebrafish. Developmental dynamics : an official publication of the American Association of Anatomists 2014;243:1646-57.
10. Sharma S, Londono D, Eckalbar WL, et al. A PAX1 enhancer locus is associated with susceptibility

to idiopathic scoliosis in females. Nature communications 2015;6:6452.
11. Miyake A, Kou I, Takahashi Y, et al. Identification of a susceptibility locus for severe adolescent idiopathic scoliosis on chromosome 17q24.3. PloS one 2013;8:e72802.
12. Justice CM, Miller NH, Marosy B, Zhang J, Wilson AF. Familial idiopathic scoliosis: evidence of an X-linked susceptibility locus. Spine (Phila Pa 1976) 2003;28:589-94.
13. Salehi LB, Mangino M, De Serio S, et al. Assignment of a locus for autosomal dominant idiopathic scoliosis (IS) to human chromosome 17p11. Human genetics 2002;111:401-4
14. Chan V, Fong GC, Luk KD, et al. A genetic locus for adolescent idiopathic scoliosis linked to chromosome 19p13.3. American journal of human genetics 2002;71:401-6.
15. Azimzadeh J, Hergert P, Delouvee A, et al. hPOC5 is a centrin-binding protein required for assembly of full-length centrioles. The Journal of cell biology 2009;185:101-14.
16. Dantas TJ, Daly OM, Conroy PC, et al. Calcium-binding capacity of centrin2 is required for linear POC5 assembly but not for nucleotide excision repair. PloS one 2013;8:e68487.:
17. Anderson RG, Brenner RM. The formation of basal bodies (centrioles) in the Rhesus monkey oviduct. J Cell Biol. 1971 Jul;50(1):10-34.

INSTITUT DE FRANCE

FONDATION YVES COTREL

POUR LA RECHERCHE EN PATHOLOGIE RACHIDIENNE

23 quai de Conti
75006 Paris
+33 (0)1 44 41 44 47
www.fondationcotrel.org