



### Animal models of cutis laxa

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## Les Animaux Modèles de la Cutis Laxa

4<sup>th</sup> Information Day on Cutis Laxa, Lyon, France, 17<sup>th</sup> September, 2011



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### Why use animal models? Pourquoi Utiliser des Animaux Modèles

- Discover the cause of cutis laxa in humans
  - Which gene is affected?
  - What are the physiological, pathological and biochemical changes that lead to cutis laxa?
- Test approaches to the treatment of cutis laxa
  - Small molecule drugs



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- \* Découvrir la cause de la Cutis Laxa chez les Humains
  - Quel gène est affecté ?
  - Quels sont les changements physiologiques, pathologiques et biochimiques qui induisent la Cutis Laxa ?
- \* Tester des approches de traitement de la Cutis Laxa
  - Très petites quantités de médicament



Yeast

### Which models to use?

- Use simplest and most economical model possible
- Move to models more similar to human to verify findings.



Worm



Human skin cells



Fruit fly



"Skin equivalent"



Mouse



Zebra fish



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### Genetic manipulation of mice



Gene knockout



Transgene



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### Quels Modèles Utiliser ?

- Utiliser les modèles les plus petits et les plus économiques possibles
- Vérifier les découvertes en utilisant des modèles de plus en plus proches de l'homme

### Manipulation Génétique de la souris

« Désactiver » le Gène    « Remplacer » le gène

### Example 1: fibulin 5 (FBLN5) Autosomal recessive cutis laxa 1B



Fibulin 5 knockout mice  
Yanagisawa et al. (2002)  
Nature 415:168-171



Human fibulin 5 mutation  
Loeys et al. (2002)  
Hum Mol Genet



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### Example 2: fibulin 4 (FBLN4) Autosomal recessive cutis laxa 1A



Fibulin 4 knockout mice  
McLaughlin et al. (2006)  
Mol Cell Biol 26:1700-1709



Human fibulin 4 mutation  
Hutchings et al. (2006)  
Am J Hum Genet 78:1075-1080



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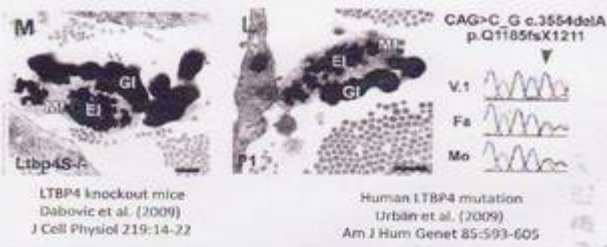
### Example 1 : Fibuline 5 (FBLN 5) Cutis Laxa Autosomale Récessive 1B

Souris avec Fibuline5 Désactivée      Mutation Fibuline5 Chez l'humain

### Example 2 : Fibuline 4 ( FBLN 4) Cutis Laxa Autosomale Récessive 1A

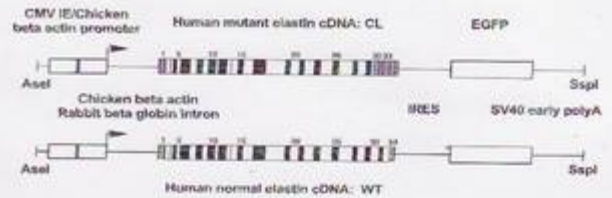
Souris avec Fibuline4 Désactivée      Mutation Fibuline4 Chez l'humain

**Example 3: latent transforming growth factor binding protein 4 (LTBP4)**  
Autosomal recessive cutis laxa 1C



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**A mouse model of autosomal dominant cutis laxa (ADCL)**



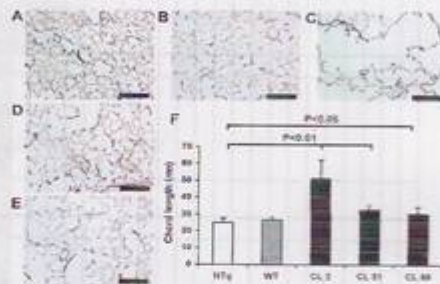
Hu et al. (2010) Matrix Biol 29:621-628

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**Exemple 3 : LTBP4**  
**Cutis Laxa Autosomale Récessive 1C**

**Une souris modèle de la Cutis Laxa**  
**Autosomale Dominante (ADCL)**

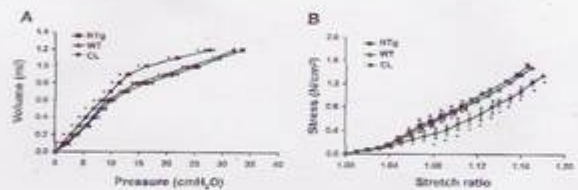
**Emphysematous lung disease in ADCL mice**



Hu et al. (2010) Matrix Biol 29:621-628

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**Increased compliance of lung tissue in ADCL mice**



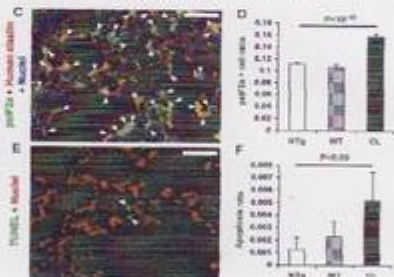
Hu et al. (2010) Matrix Biol 29:621-628

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**L'Emphysème Pulmonaire chez la souris ADCL**

**Etude du tissu pulmonaire chez la souris ADCL**

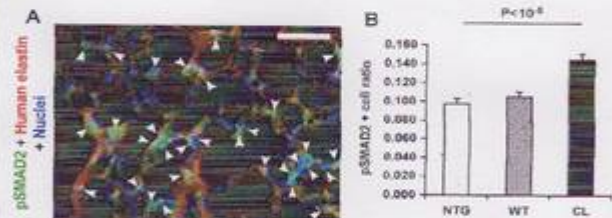
**UPR and in ADCL mice**



Hu et al. (2010) Matrix Biol 29:621-628

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**Increased TGFβ signaling in ADCL mice**



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**ADCL mouse summary**  
**Résumé de la souris ADCL**

- Replicated the emphysema component of human ADCL, but not skin laxity and aortic aneurysms.
- Mutant elastin does not fold properly and causes cell stress.
- Lung tissue in ADCL mice is looser.
- Transforming growth factor signaling is increased.

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**Genetic manipulation of zebrafish**

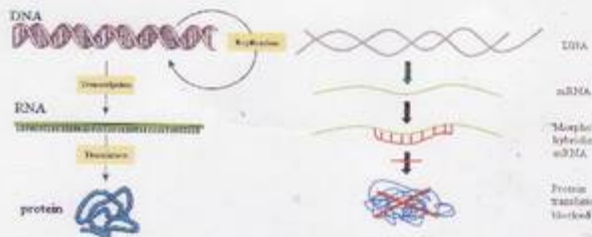


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- Réplique de l'emphysème de l'humain ADCL, mais pas de la laxité cutanée, ni des anévrismes aortiques
- L'élastine mutée ne fonctionne pas correctement et cause un stress de la cellule
- Le tissu pulmonaire de la souris ADCL est plus lâche
- Le signal de l'hormone de croissance est accru

**Manipulation génétique du Poisson-Zèbre**

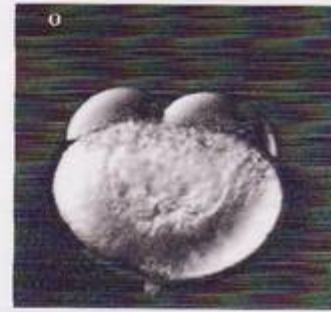
### Gene knockdown in zebrafish using morpholino



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### La Désactivation du Gène chez le poisson-zèbre

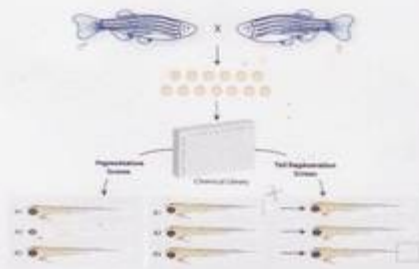
### Direct observation of zebrafish development



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### Observation directe du développement du poisson-zèbre

### High throughput small molecule drug screening



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### Test de Médicaments : Gros débit et peu de molécules

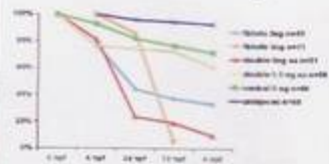
### There are 2 fibulin 4 genes in zebrafish



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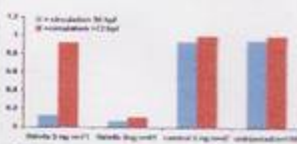
### Le poisson-zèbre a 2 gènes de la Fibuline 4

#### Survival of knockdown embryos



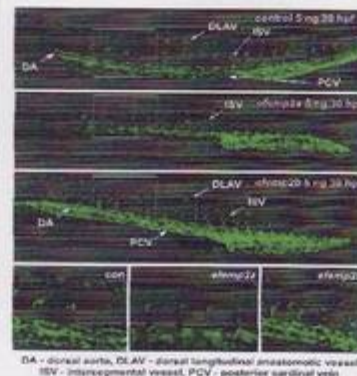
Both *fbln4a* and *fbln4b* are necessary for survival and for circulation

#### Circulation in knockdown embryos



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### Les deux, FBLN4a et FBLN4b, sont nécessaires à la survie et à la circulation

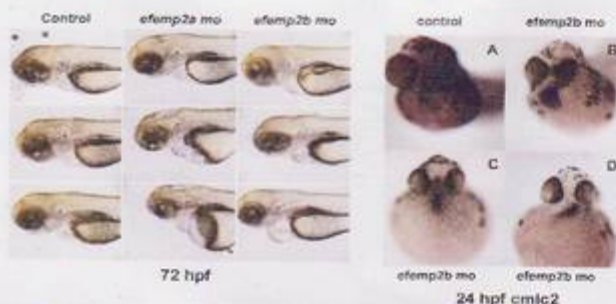


Both *fbln4a* and *fbln4b* are required for vessel lumenization

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### Les deux, FBLN4a et FBLN4b, sont nécessaires pour visualiser les vaisseaux

### Cardiac anomalies in *fbln4* deficient fish



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### Les anomalies cardiaques du poisson ayant une déficience en Fibuline 4

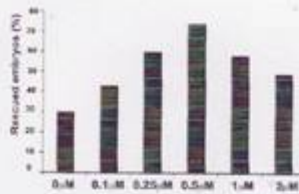
### Rescue of *fbln4b* deficiency by transforming growth factor-beta knockdown



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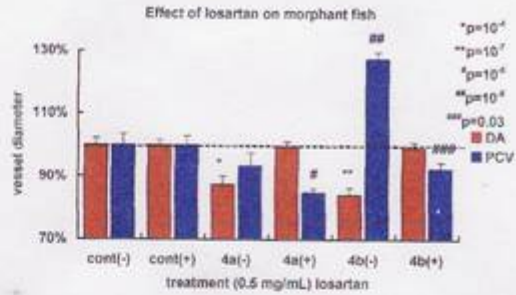
### Réparation de la déficience en FBLN4 par désactivation de l'hormone de croissance bêta

### Rescue of fibulin-4 deficiency with a chemical inhibitor of transforming growth factor



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### Losartan rescues fbln4a and fbln4b deficiency

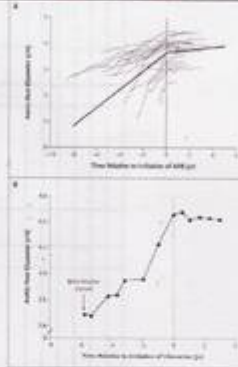


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### Réparation de la déficience en FBLN4 par Inhibiteur chimique de l'hormone de croissance

Losartan shows promise in a small, open label exploratory clinical trial of Marfan syndrome

- 18 patients with rapidly expanding aortic root
- Double blind losartan vs. atenolol trial ongoing n=600 in the US
- Several trials are ongoing in Europe



Brooke et al. (2008) N Engl J Med 358: 2787-2795

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Brooke et al. (2008) N Engl J Med 358: 2787-2795

### Le Losartan répare les déficiences de FBLN4a et FBLN4b

#### Conclusions

- We have established a zebrafish model of fibulin-4 related cutis laxa (ARCL1A).
- Transforming growth factor beta inhibition either by genetic or chemical treatment can rescue the disease associated with fibulin-4 deficiency.
- Losartan, a blood pressure medication rescues the vascular anomalies caused by fibulin-4 loss.
- Zebrafish may be a useful tool for rapid screening and identification of drugs that may correct the effects of cutis laxa mutations.

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### Le Losartan montre des possibilités dans un essai clinique pour le Syndrome de Marfan

- \* 18 patients ayant une base aortique évoluant rapidement
- \* Aux USA, essai en cours, en double aveugle, Losartan vs atenolol n=600
- \* Plusieurs essais sont en cours en Europe

- \* Nous avons créé un poisson-zèbre modèle de la Cutis Laxa FBLN4 (ARCL1a)
- \* L'inhibition de l'Hormone de croissance bêta par traitement génétique ou chimique peut réparer la maladie associée à une déficience de la Fibuline4
- \* Le Losartan, médicament pour la tension, répare les anomalies vasculaires dues à l'absence de fibuline4
- \* Le poisson-zèbre peut être un outil utile pour tester rapidement et identifier les molécules qui pourraient corriger les effets des mutations de la cutis laxa.

St. Louis



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Urban Lab



Zoell Urban



Shazma Saed



Sandeep Khatri



Golab Sher



Meghan Mac Neal



Kara Levine



Uhi-Ting Su



Maureen McGowan



Elizabeth Lawrence



Bianca

### Cutis Laxa Research Clinics



- Bi-annual clinics held in Pittsburgh, Pennsylvania, USA
- Upcoming clinic: October 21<sup>st</sup>-22<sup>nd</sup>, 2011
- Website: [www.hgen.pitt.edu/projects/cutislaxa](http://www.hgen.pitt.edu/projects/cutislaxa)
- Coordinators' E-mail: [cutislaxa@pitt.edu](mailto:cutislaxa@pitt.edu)

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