ADVANCES IN THE TREATMENT OF GENETIC DISEASES
FROM GENE SEARCH TO DRUG DISCOVERY AND CLINICAL TRIALS

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LEARNING OBJECTIVES

To point, but not to blame:

• the ignorance of what is presently available and working
• the fascination for futuristic prospects (gene therapy, stem cells)
• the trend to preconceived ideas and « a priori » among scientists
• our inability to communicate clearly and honestly
• the oversimplification of information presented by the media

To outline:

• what is already possible: “render under Caesar what is Caesar’s»
• that causality is what it is all about
• that gene identification puts us on the right track
• that replacement of a gene is not the universal panacea
• that current treatments are changing quality of life/life expectancy

The options are no longer to either recover or die but to live with a chronic disease

« The past explains our present and enlights our future » Tocqueville
Until recently, what was feasible and efficient owed virtually nothing to gene identification

"render under Caesar what is Caesar’s"

- Dietary management (early 70ies)
- Vitamin-responsive metabolic diseases
- Organ transplantations (early 80ies)
- Protein/drug engineering (early 90ies)
- Enzyme therapy (early 20ies)
- Conventional pharmacology

ADVANCES IN TREATMENT OF GENETIC DISEASES

- Dietary management (early 70ies)
  - Low protein diet: PKU, MSUD, hyperammonemias
  - Low fat diet: Hypercholesterolemias Refsum
  - Ketogenic diet: OXPHOS deficiency
  - High glucose diet: Fatty acid oxidation disorders
  - Pancreatic extracts: Cystic Fibrosis, Pearson
  - High mannose diet: CDG1b (PMI deficiency)

- Vitamin responsive metabolic diseases
- Organ transplantations
- Protein/drug engineering
- Enzyme therapy
- Gene therapy
- Conventional pharmacology
ADVANCES IN TREATMENT OF GENETIC DISEASES

- dietary management
- **vitamin/cofactor/substrate responsive metabolic diseases**
  - biotin (B8) responsive carboxylase deficiency
  - pyridoxine (B6) responsive homocystinuria
  - cobalamin (B12) responsive organic aciduria
  - tocopherol (E) responsive pseudo-Friedreich ataxia
  - carnitine responsive lipid myopathy / cardiomyopathy
  - quinone (CoQ10) responsive ataxia / OXPHOS deficiency
  - creatine: mental retardation and autistic syndromes
- organ transplantation
- protein/drug engineering
- enzyme therapy
- gene therapy
- conventional pharmacology

CREATINE DEFICIENCY IN MR AND AUTISTIC SYNDROMES

- psychomotor retardation, autistic features, seizures
- three disease genes (AGAT, GAMT, CT)
- diagnosis: NMR spectroscopy
- treatment (AGAT, GAMT): creatine (1mg/kg/d), arginine, ornithine
- improvement of epilepsy, cognitive functions, dystonia

ALL MR/ASD CHILDREN DESERVE INVESTIGATIONS ! (dg:25%)
ADVANCES IN TREATMENT OF GENETIC DISEASES

- dietary management
- vitamin responsive metabolic diseases
- Organ transplantation/neo-organes (early 80ies)
  - kidney: PKD, nephronophthisis, Alport, OXPHOS
  - liver: α1AT, biliary atresia, metabolic diseases, OXPHOS
  - heart: CMO, malformations, OXPHOS deficiency
  - bone marrow: SCID, storage diseases
  - deep brain electrostimulation: torsion dystonia (DYT1)
- protein/drug engineering
- enzyme therapy
- gene therapy
- conventional pharmacology

DEEP BRAIN STIMULATION IN GENETIC DYSTONIAS

Philippe Coubes

- bilateral implantation of electrodes by NMR stereotaxy under general anesthesia
- Medtronic® Quadripolar
- target: the postero-ventral nucleus of the Globus Pallidum
- torsion dystonia, Pentothenate kinase deficiency, Huntington chorea, OXPHOS
ADVANCES IN TREATMENT OF GENETIC DISEASES

• dietary management
• vitamin-responsive metabolic diseases
• organ transplantations
• **Protein/drug engineering** *(early 90ies)*
  - Factor VIII: *hemophilia*
  - Insuline: *diabetes mellitus*
  - GH: *growth failure*
  - Steroids: *congenital adrenal hyperplasia*
  - G-MCSF: *agranulocytosis, Pearson*
• enzyme therapy
• gene therapy
• conventional pharmacology

ADVANCES IN TREATMENT OF GENETIC DISEASES

• dietary management
• vitamin-responsive metabolic diseases
• organ transplantation
• protein/drug engineering
• **Enzyme therapy** *(early 20ies, 250K€/yr)*
  - *Fabry disease*
  - *Gaucher disease*
  - *Pompe disease*
  - *Hurler, Hunter, Maroteaux-Lamy disease*
• gene therapy
• conventional pharmacology
ADVANCES IN TREATMENT OF GENETIC DISEASES

- dietary management
- vitamin responsive metabolic diseases
- organ transplantsations
- protein/drug engineering
- enzyme therapy
- gene therapy
- Conventional pharmacology

  to rectify protein folding: vaptans, *Diabetes Insipidus*
  to re-express a fetal gene: Hydroxyurea, *Sickle cell anemia*
  to clear/chelate a toxic: benzoate, *IVA*, cysteamine, *Cystinosis*
  to lock a pathway: NTBC, *Tyrosinemia 1*
  to activate a pathway: fibrates, colchicine, *Familial Mediterranean Fever*
  to inhibit a function: bisphosphonates, *Osteogenesis imperfecta*
  to replace a function: chenodeoxycholic acid, *bile salt synthesis disorders*

TO LOCK A PATHWAY ? NTBC IN TYROSIEMIA TYPE 1

- Autosomal recessive condition (1/100,000, 1/2,000 in Quebec)
- fumarylacetoacetate hydrolase (FAAH, 15q23-q25)
- liver failure, carcinoma, tubulopathy, porphyria-like syndrome (succinyl-acetone)
- fatal outcome: liver failure 70%, carcinoma 17%
- Enormous efforts on gene therapy

- NTBC-responsive forms: 90%
Until recently, advances in the treatment of genetic diseases have owed little to gene identification and to gene therapy. They owed to biochemical elucidation of disease mechanism by the previous generation (i.e. NTBC, vaptans...). Discoveries were occasionally fortuitous ... thanks to careful non-scientist, yet talented GPs (i.e hydroxyurea, colchicine).

One does not suffer from a mutation, but from its functional consequences !! To address consequences is fair enough !

Things are changing with NGS and exome sequencing !!

- Gene identification now puts us on the right track
- Gene identification points to the target/pathway
- Gene identification inspires elegant/efficient cures
- Gene is indeed the cause, yet gene replacement is not the unique riposte !
- Gene therapy is not, and will never become a panacea
The Imagine Institute, Necker-Enfants Malades Hospital

Daytime view

ADVANCES IN TREATMENT OF GENETIC DISEASES

Things are gradually changing !!!

• Achondroplasia (*FGFR3* signaling and CNP analogue)
• Acromicric/geleophysic dysplasia, Marfan (*TGFβ* and *Ab*)
• Freidreich ataxia (*iron sulphur clusters and iron chelators*)
• Neonatal Diabetes Mellitus (*K channel and sulphonylurea*)
• Generalized pustulous Psoriasis (*IL36-Ra and anti IL-1*)
• Mycobacterial infections (*M. Tuberculosis-TB* and *IGNy*)
• Somatic gain-of-function mutations (*mastocytose and TK-I*)
• Metastatic cancers (*personalized genomics*)
ACHONDROPLASIA AND FGFR3 SIGNALING

• Achondroplasia is the most frequent cause of dwarfism (1/15,000)
• de novo mutations of FGFR3, a tyrosine kinase receptor
• constitutive activation of a key regulator of endochondral ossification

POTENTIAL CURATIVE TARGETS IN ACHONDROPLASIA

• FGFR3 TK INHIBITORS?
• ANTI-FGFR3 ANTIBODIES?
• FGFR3 RNAi?
• BLOCKADE OF DOWNSTREAM PATHWAY?
  C-type natriuretic peptide
EFFECT OF CNP ON ACHONDOPLASIA MICE IN VIVO

CNP analogue corrects the dwarfism of Fgfr3^{Y367C/+} mice (800 µg/kg, 20 days)

CNP RESCUES SIZE AND SHAPE OF GROWTH PLATE EX VIVO

H&E (x4)  
Collagen Type X  
H&E (x20)

CNP 10^{-6} M

WT  
Vehicle
CLINICAL TRIAL WITH C-type NATRIURETIC PEPTIDE (CNP)

• CNP binds to its receptor (NPR-B) and inhibits the MAP kinase pathway
• Engine analogue (39 aa) for systemic and growth plate delivery
• Circulating half-life sufficient for once daily SC injection
• Phase 1 in healthy volunteers completed
• Phase 2 in Western Europe: January 2013

THE ACROMELIC DYSPLASIA GROUP

Valérie Cormier-Daire

Short Stature
< - 3 SD
Brachydactyly
Stiff joint
Thick skin
Muscular build

Geleophysic  Acromeric  Weill-Marchesani  Myhre

ADAMTSL2  FBN1  ADAMTS 10  SMAD4
FINE TUNING OF TGFβ DOCKING IN THE EXTRACELLULAR MATRIX

ADAMTS10 (WMS)
ADAMTSL2?
FBN1 (WMS)

From Isogai et al., 2003

TGFβ ACCUMULATES IN ACROMICRIC & GELEOPHYSIC DYSPLASIA
HOW TO GET RID OF EXCESSIVE TGFβ IN THE EXTRACELLULAR MATRIX?

<table>
<thead>
<tr>
<th>TGFβ (pg/ml)</th>
<th>Control</th>
<th>GD1</th>
<th>GD2</th>
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<tr>
<td>active TGFβ</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>total TGFβ</td>
<td></td>
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</tbody>
</table>

ADAMTSL2 mutations

<table>
<thead>
<tr>
<th>TGFβ (pg/ml)</th>
<th>Control</th>
<th>GD</th>
<th>AD</th>
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</thead>
<tbody>
<tr>
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<td></td>
<td></td>
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<tr>
<td>total TGFβ</td>
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</tbody>
</table>

FBN1 mutations
TARGETING TGFβ SIGNALING IN GELOPHYSIC DYSPLASIA

- Anti TGFβ Antibody?
- Inhibitor of TGFβ signaling? angiotensin II-receptor blockers, Losartan

GD fibroblasts → Analysis of the extracellular matrix → Mouse model → Lung, Heart

ANTI-TGF β ANTIBODIES IN GELOPHYSIC DYSPLASIA FIBROBLASTS

<table>
<thead>
<tr>
<th></th>
<th>No treatment</th>
<th>10µg/ml</th>
<th>20µg/ml</th>
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<td>Control</td>
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<td></td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>GD</td>
<td></td>
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</tbody>
</table>
AN ANIMAL MODEL OF ACROMICRIC & GELEOPHYSIC DYSPLASIA

ADAMTSL2 KO mice display heart involvement, early death and growth failure

WT

Cre-CMV Adamtsl2/ff

MIMICKING mTOR INHIBITION IN TUBEROUS SCLEROSIS

- Hamartine and Tuberine normally dimerize and inhibit the mTOR pathway
- Either gene is inactivated in Tuberous Sclerosis (TS)
- The mTOR pathway is no longer inhibited
- Rapamycin, an antibiotic, mimicks the inhibitory effects of hamartine/tuberin dimers

- Rapamycin prevents epilepsy in a mouse model of TS (Ann Neurol 2008)
FRIEDREICH ATAXIA

- frequent falls
- gait ataxia
- loss of gait
- cardiomyopathy
- Diabetes (10%)

- autosomal recessive
- incidence: 1/50,000
- gene: frataxin
- GAA expansion in intron 1

Possible functions of frataxin

- iron transport
- iron handling
- ferritin
- SOD induction
- Fe-S protein synthesis
- frataxin
MRI IRON ACCUMULATION IN CNS OF FRIEDREICH ATAXIA PATIENTS

<table>
<thead>
<tr>
<th>Friedreich ataxia subjects</th>
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<tbody>
<tr>
<td>17 years</td>
<td>19 years</td>
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</table>

<table>
<thead>
<tr>
<th>Control subjects</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>20 years</td>
<td>20 years</td>
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</table>
**BRAIN IRON CHELATION IN FRIEDREICH ATAXIA AND OTHER NBIA**

- a brain-permeant chelator removes iron from dentate nuclei in FA
- benefits on gait and balance in the youngest (14yrs), still valid subjects
- unexpected clinical benefits in wheelchair-bound subjects
- coldness, foot pain, tremor, voice, incontinence
- ongoing multicentric randomized trial (*Apopharm, Toronto*)

![Graph showing variation of R2* in dentate nuclei during treatment](image)

**NEONATAL DIABETES MELLITUS AND K CHANNEL MUTATIONS**

- neonatal diabetes mellitus (DM): a life-long condition
- permanent and “transient” DM will need glucose lowering drugs
- DM + neurological issues (MR, epilepsy, hypotonia, dyspraxia)
- dominant SUR1 mutations account for a fraction of cases (~15%)
- treatable with oral hypoglycaemic agents

*The Glidkir study, Hospital Necker-Enfants Malades*

**Michel Polak**
**Mutant β Cell**

- Increased glucose
- Mutant SUR1
- K⁺
- ATP
- Stimulatory action of magnesium nucleotides (elevated ATP:ADP ratio)
- Decreased Ca²⁺ influx
- Decreased insulin secretion
- Hyperglycemia

**Mutant β Cell in the Presence of Sulfonylurea**

- Increased glucose
- Mutant SUR1
- Membrane depolarization
- ATP
- Ca²⁺
- Reduced stimulatory action of magnesium nucleotides (elevated ATP:ADP ratio)
- Stimulates insulin secretion

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**NEONATAL DIABETES MELLITUS AND K CHANNEL MUTATIONS**

**The Glidkir study, Hospital Necker-Enfants Malades**

- 19 patients (2-19yrs) on glybenclamide, insulin discontinued
- results: excellent HbA1c control (6.4%), no side effects
- improvement of tonus, concentration, but not IQ

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**Insulin pomp**

**glybenclamide**
Interleukin-36-receptor antagonist deficiency and generalized pustular psoriasis

- repeated flares with sudden onset
- diffuse, erythematous skin-eruption, rapid coverage with pustules
- high fever (39-41°C), elevated C-reactive protein levels, asthenia,


Successful treatment of GPP with the IL-1 receptor antagonist Anakinra
MENDELIAN SUSCEPTIBILITY TO MYCOBACTERIAL DISEASES & TB

Jean-Laurent Casanova

Infections by BCG and environmental Mycobacteria
Otherwise healthy children - salmonellosis (~ 1/50,000)

TWO GENETIC CAUSES IN CHILDHOOD TB

Mycobacteria

Macrophage / Dendritic Cell

NK / T Lymphocyte

IFN-γR1

IL-12

p35

p40

IFN-γR2

IL-12Rβ1

IL-12Rβ2

STAT-1

NEMO

CYBB

IRF8

Macrophage / Dendritic Cell

NK / T Lymphocyte
MENDELIAN SUSCEPTIBILITY TO MYCOBACTERIAL DISEASES

- Genetic defects of IFNγ synthesis cause mycobacterial infections (TB)
  - IFNγ given to infected children (25-50ug/m2/day)

- Genetic defects of cytokines IL17A and IL17F synthesis cause chronic cutaneomucous candidiasis
  - should respond to recombinant G-CSF and GM-CSF

- Induction of IFNα/β via TLR3 causes Herpes encephalitis (HSV1)
  - ongoing trial with recombinant IFNα in Herpes encephalitis

SOMATIC C-KIT MUTATIONS IN MASTOCYTOSIS

- a myeloproliferative disorder, cutaneous or systemic
- abnormal growth and accumulation of mast cells
- skin lesions, depression, memory loss, asthenia, pruritus
- muscle and joint pain, allergy, headache, dyspnea
- gain-of-function c-Kit mutations in a subset of BM cells

Olivier Hermine
A C.KIT- SPECIFIC TYROSINE KINASE INHIBITOR, MASITINIB, IMPROVES COGNITIVE DYSFUNCTION IN MASTOCYTOSIS

![Graph showing improvements in cognitive dysfunction](image)

Patients with pruritus score ≥6 (n=14):
- Week 0: 10
- Week 12: 5
- Week 24: 3

Depressive status (% improvement):
- Week 0: 49.9%
- Week 12: 36.9%
- Week 24: 36.9%

Patients with at least 1 flush/day (n=16):
- Week 0: 8
- Week 12: 5
- Week 24: 3

Flushes/day (% improvement):
- Week 0: 49.9%
- Week 12: 50%
- Week 24: 39.3%

Patients with Hamilton score ≥10 (n=13):
- Week 0: 12
- Week 12: 8
- Week 24: 7

Depressive status (% improvement):
- Week 0: 50%
- Week 12: 63.7%
- Week 24: 50%

Patients with pollakiuria: miction ≥8/Jour (n=7):
- Week 0: 14
- Week 12: 8
- Week 24: 7

Mictions/day (% improvement):
- Week 0: 49.9%
- Week 12: 39.3%
- Week 24: 39.3%

A C.KIT SPECIFIC TYROSINE KINASE INHIBITOR, MASITINIB, IMPROVES BRAIN PERFUSION IN MASTOCYTOSIS (ASL)

![Brain perfusion images](image)

Nathalie Boddaert

Before Masitinib | Six months after Masitinib
Bisphosphonate Cellular and Molecular Mechanisms of Action

FROM EXTREME PHENOTYPES TO COMMON DISEASES

Before treatment

After treatment

OI-III
9 yrs

Tx 2.3 yrs
Ct.Wi: 187 µm ⇒ 534 µm

OI-IV
10 yrs

Tx 2.4 yrs
Ct.Wi: 449 µm ⇒ 1096 µm
n = 45

Rauch et al, JCI 2002
PERSONALIZED GENOMICS IN TREATMENT OF METASTATIC CANCERS

- **aim**: to identify genomic signatures in an individual tumor and enable treatment by re-purposed or newly developed drugs
- **tools**: NGS and advanced bio-informatics
- **endpoint**: tumor reduction (imaging) and clinical response in terms of survival and quality of life
- **population**: relapsed, refractory or newly diagnosed cases of metastatic disease, with no known curative therapy
- **all patients/legal guardians must sign an IRB-approved form**

*Shah et al, Nature 2012*
ADVANCES IN TREATMENT OF GENETIC DISEASES

Conclusion I

Many questions and concerns for genetics of the future !!!

• are clinical trials on very small series/single cases feasible?
• how to proceed from extremely rare to common diseases?
• how should academics and industry cooperate to maximize fertile interactions for fast drug discovery?
• yet, will our options still remain economically and ethically acceptable?
• how shall we preserve our values of frugality and solidarity?

Conclusion II

Diagnosis and causality is what it is all about…

The challenge is not the Promethean dream to cure all diseases, but rather to identify what is possibly treatable…

One should beware of single thought, dogmatisms, and certainties. Science is pragmatism, not ideology…

«One cannot order a discovery…»  Lavoisier

Let us learn from our past mistakes, keep our eyes open and not put all our eggs into the same basket …

THANK YOU !!
Mast cells and Neurofibromatosis
Simplified pathways of human MC differentiation

**Tissues**

- **MG_C**
- **MG_TC**
- **MC**

**Circulation**

- **IL-4**
- **SCF**

**Survival:**

- SCF
- NGF
- IL-4
- IFN-γ

**Bone marrow**

- **Stem Cell**
- **CD34+**

- **CD34+ c-kit high FceRI neg CD13+**

**ISG15: A NEW IFN-G-INDUCING CYTOKINE**

**Mycobacteria**

- **ISG15**
- **IL-12 p35**
- **p40**
- **CD40**
- **IFN-gR1**
- **IFN-gR2**

**Mononuclear Phagocytes & T Cells:** ‘adaptive’ IL-12-IFN-g

**Polymorphonuclear Phagocytes & NK Cells:** ‘innate’ ISG15-IFN-g
Latent TGFβ Binding Protein 1 (LTBP1) is a partner of ADAMTSL2.