# RARE DISEASES
# IN SOUTH - EASTERN EUROPE
# SECOND MEETING

# РЕТКИ БОЛЕСТИ
# ВО ЈУГОИСТОЧНА ЕВРОПА
# ВТОР СОСТАНОК

MACEDONIAN ACADEMY OF SCIENCES AND ARTS (MASA)
SKOPJE, MACEDONIA
RESEARCH CENTER FOR GENETIC ENGINEERING
AND BIOTECHNOLOGY "G.D.EFREMOV"

МАКЕДОНСКА АКАДЕМИЈА НА НАУКИТЕ И УМЕТНОСТИТЕ (МАНУ)
СКОПЈЕ, МАКЕДОНИЈА
ИСТРАЖУВАЧКИ ЦЕНТАР ЗА ГЕНЕТСКО ИНЖИНЕРСТВО
И БИОТЕХНОЛОГИЈА „Г.Д.ЕФРЕМОВ"

Skopje, November 16, 2013
Скопје, 16 ноември 2013



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**Nada Pop-Jordanova** (Skopje, Macedonia)

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***Конгресот ќе се одржи во МАНУ, сместувањето е во**
Hotel Continental, bul. Aleksandar Makedonski b.b., 1000 Skopje, Macedonia,
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***The Congress will be held at MASA, Skopje; accommodation at the**
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**ЈАЗИК / LANGUAGE**
Official language is English.

## SECOND MEETING ON RARE DISEASE IN SOUTH EASTERN EUROPE

## MACEDONIAN ACADEMY OF SCIENCES AND ARTS SKOPJE, MACEDONIA

### FRIDAY, NOVEMBER 15

**20.00- Get together party, Hotel Kontinental Skopje**

### SATURDAY, NOVEMBER 16

### MACEDONIAN ACADEMY OF SCIENCES AND ARTS

**09.00-12.00 Plenary session – Rare Diseases in SEE**

**Moderators:** Ilija Vaskov, Zvi Laron, Zoran Gucev, Nadica Pop-Jordanova

09.00–09.30 **Welcome and opening**

**Nikola Todorov, Minister of Health, Macedonia**

**Vlado Kambovski, President of the Macedonian Academy of Sciences and Arts**

**Momir Polenakovic, Director Research Center for Genetic Engineering and Biotechnology "G.D.Efremov", Macedonian Academy of Sciences and Arts**

09.30–10.00 **Zvi Laron** (Tel Aviv, Israel)
Patients with Laron syndrome are protected from cancer

10.00–10.30 **John Chaplin** (Goteborg, Sweden)
Psychosocial and cognitive effects of growth hormone treatment

10.30-10.50 **Nada Pop-Jordanova** (Skopje, Macedonia)
Some psychological specifics in children and adolescents with chronic illnesses

10.50.11.10 **Liljana Saranac** (Nis, Serbia)
Growth hormone deficiency in some rare diseases

11.10-11.30 **Zoran Gucev, Velibor Tasic** (Skopje, Macedonia)
Rare diseases, new molecular insights gained

11.30-11.50 **Snezana Bojcin, Baba Slavica Stojanovska, Vesna Aleksovska** (Macedonia)
M. Hunter, M. Gaucher and Cystic Fibrosis – stories from parents and patients

11.50-12.20 **Hans-Joachim Seitz** (Hamburg, Germany)
Thyroid Hormone Resistance – Molecular Basis, Clinical Picture

12.20-12.40 **Discussion**

12.40-14.00 **Lunch Break**

## Genetics&Varia

Moderators: Ilija Filipce, Velibor Tasic, Katrin Kollman, Dijana Plasevska Karanfiska

14.00-14.30 **Vukasin Andric** (Zagreb, Croatia)
Enzyme Replacement Treatment (ERT) in Gaucher disease

14.30-15.00 **Katrin Kollmann** (Hamburg, Germany)
The lysosomal storage disorder mucolipidosis type II - Pathomechanisms and therapeutic strategies.

15.00-15.20 **Velibor Tasic** (Skopje, Macedonia)
Sindromatic CAKUT

15.20 -15.40 **Tatjana Jakovska-Maretti, S.Fustik** (Skopje, Macedonia)
Cystic Fibrosis: Prevalence of low bone mass and vitamin D deficiency in pediatric and adult CF patients in R.Macedonia

15.40-16.00 **Dijana Plasevska Karanfiska** (Skopje, Macedonia)
Beta thalassemia intermedia

16.00-16.20 **Aco Kostovski** (Skopje, Macedonia)
Metabolic liver disease: a diagnostic and treatment challenge -experience from Gastroenterohepatology Department

16.20-16.40 **Marina Krstevska-Konstantinova** (Skopje, Macedonia)
Genetic background of precocious puberty

16.40-17.00 **Snezana Jancevska, Mile Kitanovski, Ilija Kirovski**
(Skopje, Macedonia)
Emanuel syndrome – clinical and molecular analysis

17.00-17.30 **Discussion**

17.30-18.00 **Momir Polenakovic, Zoran Gucev, Velibor Tasic**
(Skopje, Macedonia)
Conclusions and Take home message

*Reviews in Endocrinology and Metabolic 2013;1:in press*

## LARON ZVI

*Laron Zvi MD, Steuerman Rachel MD, Shevah Orit MSc, Kauli Rivka MD*
*Endocrinology & Diabetes Research Unit, Schneider Children's Medical Center, Sackler School of Medicine, Tel-Aviv University, Israel*

### PATIENTS WITH LARON SYNDROME ARE PROTECTED FROM DEVELOPMENT OF CANCER EVEN IF TREATED WITH IGF-I

**Running Title:** Cancer protection in Laron syndrome

**Abstract:** In accordance with the link between increased GH and IGF-I secretion and cancer, we found that homozygous patients with Laron syndrome (severe GH insensitivity) and low to undetectable serum IGF-I are protected from developing cancer even if treated with IGF-I to enhance linear growth.

**Keywords:** Laron syndrome, GH receptor, IGF-I and cancer, GH insensitivity; cancer
Growth hormone and IGF-I are recognized cancer stimulating hormones. To test this hypothesis we studied patients with Laron syndrome (LS) who by genetic defects in the GH receptor lack since conception GH and IGF-I activity. Collecting data from other clinics in addition to our LS 68 patients we found that none of 234 homozygous patients with LS developed cancer during lifetime even if treated by IGF-I. In contradistinction their heterozygous family members are not protected. In Ecuador with 90 patients our findings were confirmed.

Ref.
Steurerman R, Shevah O, Laron Z. Congenital IGF-I deficiency tends to confer protection against postnatal development of malignancies. Eur J Endocrinol. 2011;164:485-489

Reviews in Endocrinology and Metabolic 2013;1:in press

# Patients with Laron Syndrome are protected from development of cancer even if treated with IGF-I

Laron Zvi MD, Steuerman Rachel MD, Shevah Orit MSc, Kauli Rivka MD

Endocrinology & Diabetes Research Unit, Schneider Children's Medical Center, Sackler School of Medicine, Tel-Aviv University, Israel

Running Title: Cancer protection in Laron syndrome

## Abstract

In accordance with the link between increased GH and IGF-I secretion and cancer, we found that homozygous patients with Laron syndrome (severe GH insensitivity) and low to undetectable serum IGF-I are protected from developing cancer even if treated with IGF-I to enhance linear growth.

**Keywords:** Laron syndrome, GH receptor, IGF-I and cancer, GH insensitivity; cancer

## Introduction

Cancer cells are characterized by their capacity for self-sufficient proliferation, refractoriness to growth inhibitory signals, resistance to apoptosis and capacity to recruit angiogenesis (1). The causes of cancer include genetic predisposition, gene-environment interactions and infections agents. Among the risk factors are hormones including pituitary growth hormone (GH) and insulin like growth factor I (IGF-I) (2-4).

In 1966 we described a new disease resembling congenital isolated GH deficiency (c1GHD) (5) but which surprisingly was found to be characterized by high serum levels of GH and low to undetectable levels of IGF-I (6). The patients originated from the Middle East and most belonged to consanguineous families (7). Subsequently more patients were diagnosed in various parts of the world. (5), and the disease was coined by William Daughaday Laron type dwarfism, and changed subsequently to Laron syndrome (LS, OMIM#262500).

In 1983 we showed that that liver membranes of LS patients could not bind 125I-hGH explaining the etiology of this disease as insensitivity (resistance) to GH (8), subsequently we and others demonstrated that the resistance to GH was due to deletions (9) or mutations in the hGH receptor (5, 10).

## Aim of Study

Having a population with an inability to generate IGF-I we considered it to be the right model to test, the relationship between IGF-I deficiency and cancer.

We hypothesized that if indeed GH/IGF-I promotes malignancy: patients with congenital IGF-I deficiency as exemplified by Laron syndrome should be cancer free.

## Subjects and Methods

To test this hypothesis we analyzed the medical charts of our 68 LS patients, sent a questionnaire to medical clinics known to follow patients with LS and screened the medical literature for published patients (11-13).

We were able to collect data on 234 patients with LS, adding recently 4 new patients to those included in the report by Steuerman et al (14). Dr Guevara-Aguirre who follows 99 patients in Ecuador expressed the wish to analyze their patients separately (personal communication).

It is assumed that both groups together amounting to 334 patients represent about 2/3 of all Laron Syndrome patients in the world. Sixty-four patients in the pediatric age group had been

treated with IGF-I for many years; so were 5 adult patients but only for one year (5, 7).
We were also able to obtain data on 339 first and second degree relatives.

## Statistics

The collected data were analyzed using the BMDP statistical software (14). One-way and two-way ANOVA were used to analyzed continuous variables (age) and as for discrete variables (diagnosis/ type of malignancy). $X^2$ test and Fisher's exact test were used. A P value <0.05 was considered statistically significant.

## Results

The study was performed in two stages:
The first part performed during the years 2004-2006, led to the announcement of the preliminary and exciting results in 2005 at a cancer meeting in Taormina (15) namely that none of the LS patients had developed cancer their age ranging from 3-78 years with a mean age of 32.3 years in our cohort and 16 years in the US and European one. These findings in 169 patients with Laron syndrome and 250 relatives were published in 2007 (16). We continued to collect data to ascertain these findings and published the second stage early in the year 2011 (14). The study was updated until August 2013. Table 1, which also includes the findings of the Ecuadorian groups (17).
It is evident that none of the homozygous LS patients many of them adults, even old, had developed any malignancy whereas their heterozygote 1$^{st}$ degree relatives have.

## Discussion

Our findings that homozygous patients with Laron syndrome are protected from developing cancer have been confirmed by Guerara-Aguirve et al (17) who surveyed 99 patients with LS in Ecuador. One of their LS patients who developed ovarian carcinoma was a double heterozygote. The mechanism which protects the patients with Laron syndrome is so far undetermined. Their 1$^{st}$ degree heterozygote family members (parents and siblings) who are not protected from cancer do not resemble phenotypically LS patients (6, 7). The cause must be related to the fact that the heterozygote subjects produce active growth hormone and IGF-I. The conclusion is that only complete congenital absence of IGF-I induces a cancer protective mechanism.
We (18) and probably others (17) are taking on the challenge to solve the riddle which should lead not only to a better understanding of the link between IGF-I and cancer but hopefully to improved and novel treatment

## References

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2) Werner H, Bruchim I. The insulin growth factor-1 receptor as an oncogene. Arch Phyiol Biochem. 2009;:115:58-71

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4) Frasca S, Pandini G, Sciacci L, Pezzino V, Squatrito S, Belfiore A, Vigneri R. The role of insulin receptors and IGF-I receptors in cancer and other diseases. Arch. Physiol. Biochem. 2008;114:23-37

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10) Shevah O, Laron Z. Genetic analysis of the Pedigrees and Molecular Defects of the GH-Receptor Gene in the Israeli Cohort of Patients with Laron Syndrome. Pediatr. Endocrinol Rev. 2006; 3 (Suppl 3): 489-497. Erratum Pediatr. Endocrinol Rev. 2007;5:470

11) Rosenfeld RG, Rosenbloom AL, Guevara-Aguirre J. Growth hormone (GH) insensitivity due to primary GH receptor deficiency. Endocr Rev. 1994;15:369-390

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14) Steuerman R, Shevah O, Laron Z. Congenital IGF-I deficiency tends to confer protection against postnatal development of malignancies. Eur J Endocrinol 2011;164:485-489

15) Shevah O, Laron Z. Patients with congenital deficiency of IGF-I seem protected from the development of malignancies: A preliminary report. Growth Hormone & IGF Res. 2007;17:54-47

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18) Lapkina L, Laron Z, Werner H, Genome-wide analysis of cancer protection pathways in Laron Syndrome patients. Abstr# P01-22. Growth Hormone & IGF Research. 2012;22:S39

**Table 1**

**Prevalence of malignancies in Laron syndrome patients and their first degree relatives**

| **Subject** | | | |
|---|---|---|---|
| | **N** | **N** | **%** |
| Laron syndrome | 234 | 0 | 0 |
| 1st degree relatives | 218 | 17 | 8.3 |

**JOHN CHAPLIN**
*(Goteborg, Sweden)*

## PSYCHOSOCIAL AND COGNITIVE EFFECTS OF GROWTH HORMONE TREATMENT

**Abstract:** The aim of this presentation is to evaluate effects of growth hormone (GH) treatment on the psychosocial and cognitive characteristics of short-stature children. **In this presentation some background data will be presented but I will focus on the results from a longitudinal clinical trial of GH treatment carried out in Sweden since year 2000. 9**9 referred pre-pubertal non-familiar short-stature children (32 GH deficiency; 67 idiopathic short stature) aged 3–11 years, were randomized to fixed or individual GH doses and their parents completed questionnaires and cognitive testing at baseline (BL) and after 3, 12, 24 and 48 months. **Results:** At BL, children showed higher levels of internalizing behaviour ($p< 0.001$), lower levels of externalizing behaviour ($p< 0.006$) and self-esteem ($p< 0.001$) compared to reference values. During GH treatment, behavioural measures ($p< 0.001$) and depression ($p< 0.01$) changed towards the mean of the population within the first 3 months and remained improved to 24 months. Self-esteem improved at all time-points ($p< 0.001$), and in all subgroups, as did well-being dimensions *stability* and *mood* ($p< 0.05$). **Conclusion:** GH treatment of pre-pubertal short children significantly improved behavioural, depression, and psychosocial evaluations over a 4-year period of GH treatment. Most change occurred within the first 3 months, which highlights this short period as important not only for growth and metabolic changes but also for behaviour and psychosocial improvements following GH treatment.

Nov 2013

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# Psychosocial and cognitive effects of growth hormone treatment

John Eric Chaplin
PhD Leg.psyk. AFBPS
Gothenburg Pediatric Growth Research Center
The Queen Silvia Childrens Hospital
The Sahlgrenska Academy at the University of Gothenburg

Sahlgrenska akademin

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## Short stature due to GH deficiency

- The effects of growth hormone (GH) treatment can be profound:
  - –The child grow taller towards a normalised height,
  - –Release from social or psychological burdens attributable to short stature.
    - –changes in expressive and internalised behaviours,
    - –changes in well-being and self-esteem.
- Height gain, although often the primary outcome of GH treatment can also be taken as an indicator of social and psychological changes in the individual.

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## *Are short adults & children psychologically worse off than those with age-appropriate height?*

- **QoL**
  - – Christensen (2007) - Adult population - height-related differences in QoL.
  - – Appelman-Dijkstra (2013) - Systematic review - adults – significant relationship between height and QoL.
  - – Spielhagen (2011) – Adults – positive effect on QoL
- **Social immaturity**
  - – Lee et al (2009) - Child population (11 years and older). No differences in social, emotional or behavioural outcomes, including depression, optimism, social support or victimisation, by either self- or teacher-reported findings
- **Self-esteem**
  - – Downie (1997) - Child /Adolescents – Wessex Growth study. The short children did not differ significantly from the controls on measures of self-esteem, self-perception, or behaviour.
  - – Chaplin (2011) - Child – clinic population – Self-esteem improvement over first two years
- **Bullying**
  - – Voss (2000) - Adolescents - Wessex Growth study - short children are more likely to be bullied than their taller peers and report more social isolation.
- **Juvenilization**
  - – Ulph (2004) - Adolescent/Adult – Wessex Growth study. No evidence that stature per se significantly affected education and employment, love relationships, friendships, coping, or psychological well-

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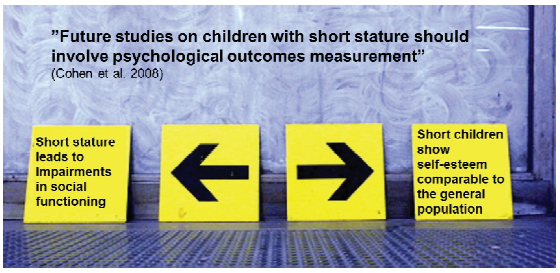


**With currently available data, it is difficult to generalize on the impact of short stature on psychosocial adaptation.**

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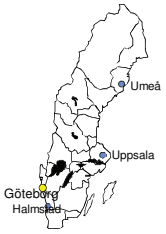
## Gh-dose QoL

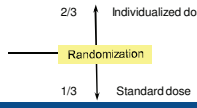
National multicentre pre-pubertal randomized

*Kriström B et al, J Clin Endocrinol Metab 2008*




| | Girl | Boy | Total | Mean age at Start |
|---|---|---|---|---|
| ISS | 19 | 48 | 67 | 7.2 years |
| GHD | 10 | 22 | 32 | 7.5 years |
| Total | 29 | 70 | 99 | 7.4 years (SD 2.06) |

Categorization ISS/GHD, spontaneous GHmax during a 24h-sampling with a cut off 32 mU/L corresponding to 22,6 mU/L using the monoclonal antibody.

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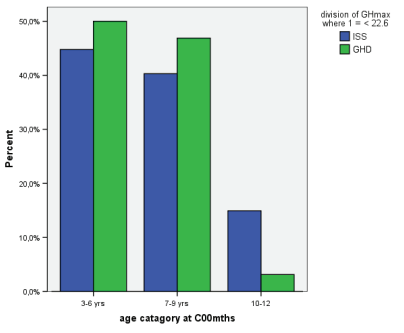
♂ 70

♀ 29

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## Psycho-social questions

- Do short children have emotional or behaviour problems?
- Can we identify changes in association with treatment?
  - –Self-esteem - ("I Think I Am" questionnaire)
  - –Depression - (Birelson)
  - –Behaviour - (CBCL, Connors)

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## *I think I Am* – self-esteem

- Two age versions 8-12 and13-16 Likert type scales 32 and 72 questions with 5 domanins
  1. Physical capacity,
  2. Abilities and talents,
  3. Psychological well-being,
  4. Relations with the family,
  5. Relations with other people.
  - –Total score

Ref: Normer och tillförlitlighet som finns i Jag tycker jag är (Quoniam-Bergerstam, 1985, 1999).

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## Depression – Self-completion (Birelson)

- 18 questions rating frequency of depressive symptoms
- Exemple statements:
  - I look forward to things as much as I did before
  - I like to go out with my friends
  - I have loads of energy
  - I like to talk with my parents and my family
  - I feel very alone
- A score of15 points or higher is considered to have symptoms of depression.

Birleson P (1981a) Depression self-rating scale (DS-RSS). Royal Children's Hospital, Thornbury Road, Parkville, Victoria 3052, Australia
Birleson P (1981b). The validity of depression disorders in childhood and the development of a self-rating scale: a research report J Child Psychol Psychiatry 22:73–88



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## Child Behaviour Checklist (CBCL)

• Behaviour and emotional problems in children and young people between the ages of 4 and18 years.

Standardised parents questionnaire. Consisting of:
- 118 quesitons,
- 8 domains,
- 2 problem areas
  - Internalised problems - (fear, inhibited behaviour)
  - Externalised problems - (aggression, antisocial behaviour)



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GH dose Psycho-Social Results



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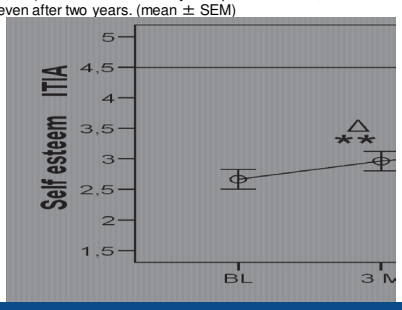
**Self-esteem – significant improvements** (p <0,01, p <0,001, p <0,001) from baseline to every time point. However, it did not reach the norm point even after two years. (mean ± SEM)






## There are changes during GH-treatment

| I Think I Am - self-esteem questionnaire | N | Mean base line | *Mean 24 mths* | Sig |
|---|---|---|---|---|
| Physical capacity, | 60 | 4.26 | 4.73 | 0.09 |
| Abilities and talents, | 50 | 3.86 | 3.78 | 0.56 |
| Psychological well-being | 61 | 5.52 | 5.55 | 0.59 |
| Relations with family | 57 | 3.03 | 4.25 | 0.10 |
| Relations with others | 54 | 3.80 | 4.94 | 0.14 |
| Total score | 87 | 5.67 | 6.18 | **0.050** |



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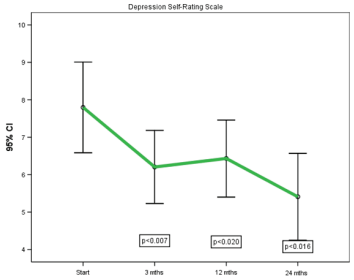
## Short stature children do not have emotional problems.




Birelson questionnaire

Clinical indication of depression = 13/15 points.



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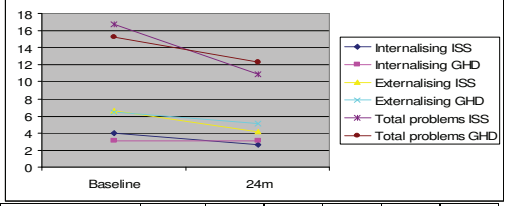
Child Behaviour Checklist




| Parents | GHD | | | ISS | | |
|---|---|---|---|---|---|---|
| CBCL | baseline | *24 m* | Sig | baseline | *24 m* | Sig |
| Internalising | 3.15 | 3.13 | 0.91 | 4 | 2.63 | **0.006** |
| Externalising | 6.48 | 5.15 | 0.16 | 6.63 | 4.13 | **0.004** |
| Total | 15.2 | 12.4 | 0.05 | 16.8 | 10.9 | **0.001** |



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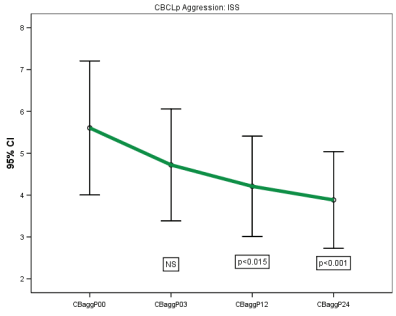
## Aggression problems ISS – CBCL



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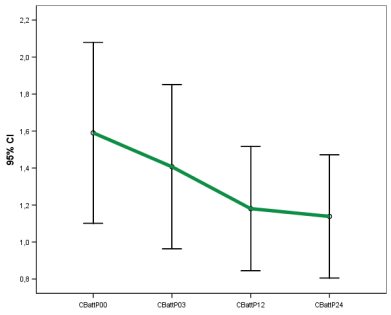
## Attention problems ISS – CBCL



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## Conclusions about psycho-social effects

- Improved self-esteem, well-being and lower depression scores.
- Behaviour problems normalised.
- Perception of normal height achieved within the first 3 months of treatment.



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## Cognitive functioning Questions

- Do children with short stature have age appropriate IQ?
- Are there changes in IQ during GH-treatment?
  - –Total IQ (Verbal IQ, Spacial IQ)
  - –Perceptuel organisation
  - –Are there children who benefit more than others?



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## Cognitive functioning

- GH & IGF-1 receptors in the brain
- - but is there any evidence of a congnitive effect?



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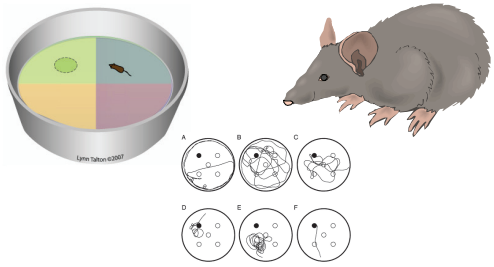
Rats i Morris water labyrinth, a spacial memory test.




CNS Targets for GH and IGF-1: emphasis on their regulation in relation to cognitive processes. Le Greves. Dissertation 2005 Uppsala



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Rats treated with GH demonstrated improved abilities in the Morris water labyrinth.

The Hx rats performed significantly better in the MWM on the second and third day of training compared to control animals (Figure 11). After further training the untreated animals performed equally good as the GH-treated group of rats.

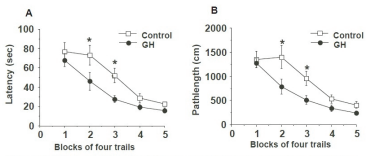


*Figure 11.* Effects of GH- and vehicle treatment in Hx rats on performance in the MWM behavioural task. Panel A shows the escape latency (time to reach the platform in seconds), and panel B the length of swim path (distance in centimetres) during acquisition trails. *P<0.05, **P<0.01 vs. controls, ANOVA and post-hoc *t*-tests.

CNS Targets for GH and IGF-1: emphasis on their regulation in relation to cognitive processes. Le Greves. Dissertation 2005 Uppsala



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## Short Stature and Functional Impairment A Systematic Review

**Table 3. Visual-Motor Skills Among Children With Short Stature**

| Source | Study Sample[a] | Mean Height | Measure | Results[b] | Sample Source | Quality[c] |
|---|---|---|---|---|---|---|
| **ISS** | | | | | | |
| Abbott et al,[5] 1982 | 11 | 81-132 cm | Bender Visual Motor<br>Beery Visual Motor Integration | **–2 y 1 mo**[d]<br>**–3 y 8 mo**[d] | Clinic | B |
| Young-Hyman,[24] 1986 | 27 | . . . | Bender Visual Motor | **–2 y 6 mo**[d] | Clinic | C |
| **GHD or MHD** | | | | | | |
| Siegel and Hopwood,[19] 1986 | 42 | <–2 SDS | Bender Visual Motor<br>Score <16th percentile and ≥4 brain injury indicators | 26% | Clinic | B |

Abbreviations: GHD, growth hormone deficiency; ISS, isolated short stature; MHD, multiple hormone deficiency; SDS, standard deviation score (standard deviations from population mean height); ellipses, no data.
[a]No study evaluated normal height controls.
[b]Results in bold were significantly different from normal population.
[c]A = Good quality, least bias; B = fair quality, susceptible to some bias; and C = poor quality, substantial bias likely.
[d]Discrepancy between chronological age and age equivalence.

Wheeler et al. (2004) Arch. Pediatric Adolescent Medicine, 158:236-243



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## Psychological effects in GHD adults treated with GH N=18

| | Baseline | Efter 6 månader | Wilcoxon p values |
|---|---|---|---|
| **Verbal intelligens** | | | |
| Digit Span Test: | | | |
| – Forwards | 5.5±0.5 | 6.1±0.6 | Ns |
| – Backwards | 5.2±0.4 | 5.3±0.5 | Ns |
| attention/concentration | | | |
| **Spatialt intelligens** | | | |
| Digit Symbol Test: | | | |
| – Raw score* | 46.4±2.8 | 51.5±3.1 | 0.01 |
| – Time to complete | 156.1±16.4 | 127.4±9.2 | 0.001 |
| Digit Symbol - Coding | | | |

Lasaite et al. (2004) *Medicina (Kaunas) 2004; 40(8):740-744*

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# IQ

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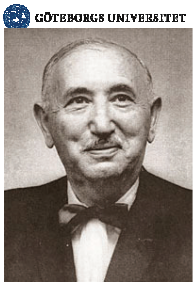

*David Wechsler (1896-1981)*

"Intelligence is the aggregate or global capacity of the individual to act purposefully, to think rationally and to deal effectively with his environment (Wechsler, 1944, p. 3)."

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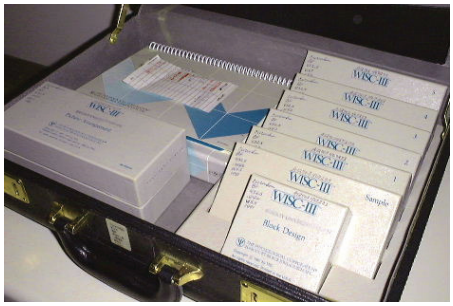
WISC-III: Wechsler Intelligence test for children



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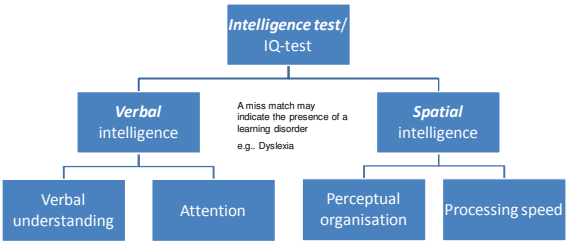
## Cognitive functioning

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## Två-poängsuppgifter och svarsexempel

6. *På vilket sätt är ett ÄPPLE och en BANAN lika?*

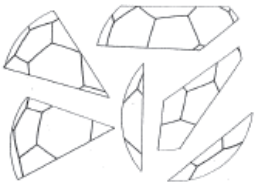
*2 poäng* — *Ett svar som anger att båda är frukter*
*(Om barnet säger att "banan är ett bär" ställ en följdfråga.)*

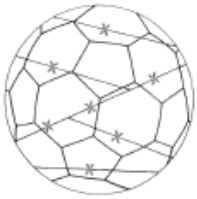
*1 poäng*
- Båda är mat (söta)
- Båda är ätbara (man äter dem)
- Man kan använda båda att göra paj och kakor av
- Båda har skal
- Båda växer på träd
- Båda är nyttiga (hälsosamma; ger energi; har kalorier; har vitaminer)
- Det nyttiga är inuti

*0 poäng*
- Bra att äta (F)
- Båda är runda (långa; smala; gula)
- Samma sorts skal
- De är mjuka
- Båda har fruktsaft (juice)
- Smakar lika
- Man äter dem till efterrätt
- Båda är bär

Börja ta tid. Tillåten tid: 180 sekunder. *Observera:* Nämn inte vad figuren ska föreställa!

Barn





Testledare Antal fogar = 7

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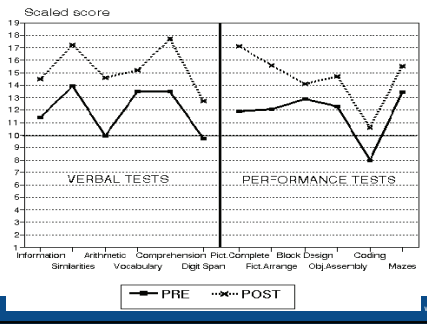
## Cognitive functioning: Intelligence tests measure the individuals ability to solve unfamiliar problems.

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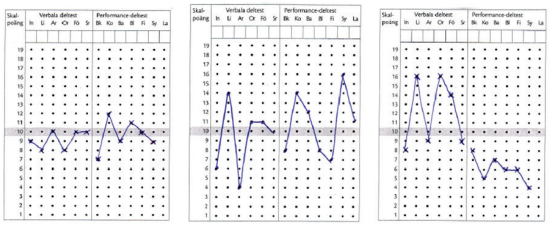
## Three examples all with the same total IQ score of 97

VIQ=95 PIQ=98 VIQ=95 PIQ=98 VIQ=117 PIQ=74



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GH dose Cognitive Results

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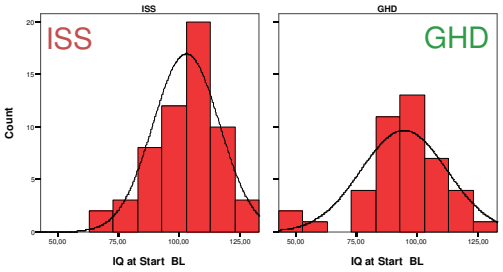
IQ at study start.

Short stature children appropriate IQs for age.

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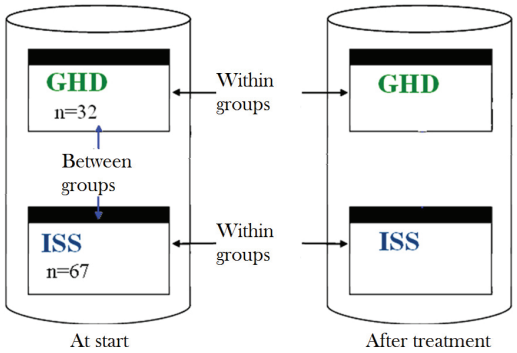


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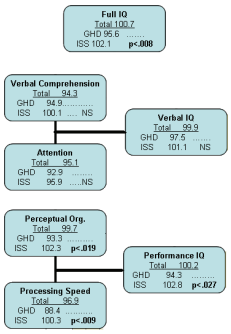
**Before treatment (between groups)**




There are some differences between GHD and ISS.

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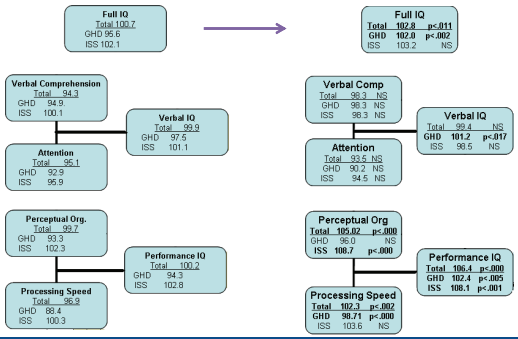
**After treatment (within group analysis)**

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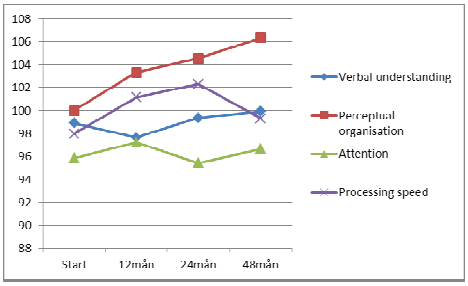
Perceptual organisation improves.

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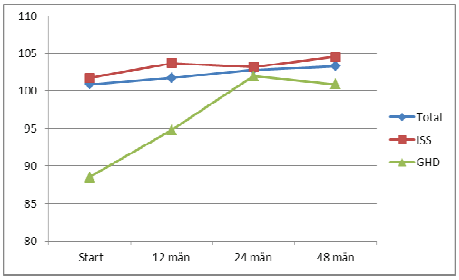
There is an improvement in total IQ over the period.

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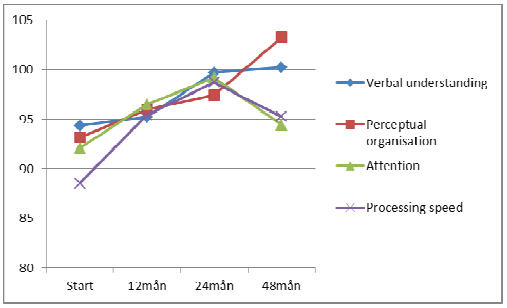
GHD-group

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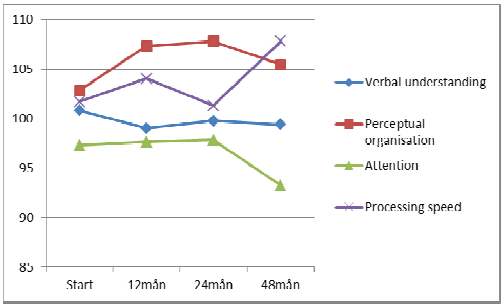
ISS-group

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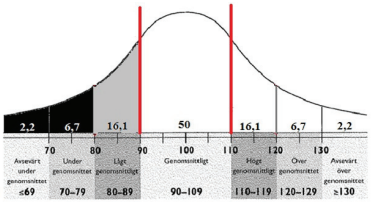
Are there some children who gain more than others?

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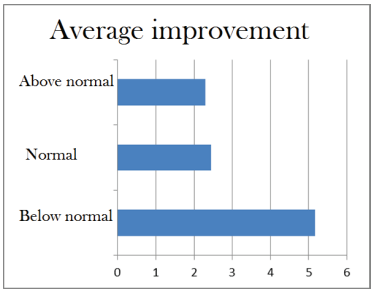
IQ improves more in those with the lowest IQ scores

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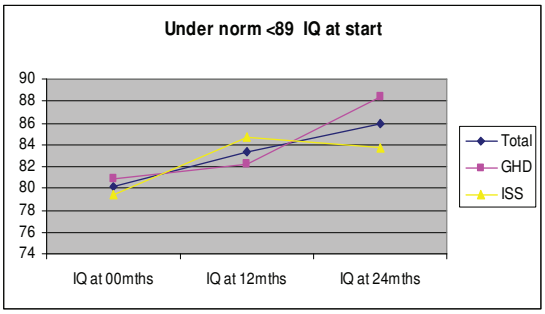
Children with the lowest IQ scores increased IQ most

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## Conclusions for the cognitive part

- IQ improved
- Performace IQ increases most
- IQ improved mostly in those with low IQ at start

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## General conclusions

- Relative to before treatment, short children receiving GH can be described to have improved psycho-social variables, cognition and quality of life.
- However, the pattern of problems experienced by short children is individual and will change with the age of the child and exposure to challenges in their social and physical environment.

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**NADA POP-JORDANOVA**
*Macedonian Academy Of Sciences And Arts*

## SOME PSYCHOLOGICAL SPECIFICS IN CHILDREN AND ADOLESCENTS WITH CHRONIC ILLNESSES

**Abstract:** Significant number of children suffers from chronic diseases, which impose careful adjustment, coping and active role of all involved in the treatment. Psychological problems in that population are increased by the long duration of procedures, specific diet and low physical activity. In this study different psychometric instruments are used to evaluate the psychological characteristics of children and adolescents suffering from cystic fibrosis, juvenile rheumatoid arthritis, diabetes mellitus and cancer. The obtained results are compared between groups as well as with control. Children with cystic fibrosis and neuroticism appeared to be more aggressive, while those with juvenile rheumatoid arthritis and cancer more anxious and depressed in comparison with control. The personality profiles in adolescents with cystic fibrosis and cancer is similar showing Hs-D-Hy picks. Manifest depression is found only in the group with cancer and occasionally in cystic fibrosis. The profiles for diabetics showed emotional instability as well as some psychopathological traits. Generally, the psychological functioning in all children is not so impaired. Multidisciplinary teamwork is needed for overcoming the arising psychological problems as well as to obtain good quality of life.
**Key words:** chronic diseases, children, psychology.

## Psychological specifics in children and adolescents with chronic illness

NADA POP-JORDANOVA
MACEDONIAN ACADEMY OF SCIENCES AND ARTS

## Introduction

- A significant number (7.5-10%) of children actually suffer from chronic diseases
- There are many different types of chronic diseases. Some are present at birth, while others may develop at the later stage during infancy or childhood.
- In our country the main chronic diseases among children and adolescents are **asthma, diabetes, cancer, cystic fibrosis** and **epilepsy**

## Criteria for chronic condition (O'Halloran):

- duration at least 6 months
- pattern of recurrence or deterioration
- poor prognosis
- produce consequences or sequels that impact on the individual's quality of life.

## Chronic diseases in childhood are significant for several reasons:

- they threaten the normal child's development,
- the care can be extremely complex and require a combination of medical and other services,
- the care is very costly over a long period of time.
- consequently, the socioeconomic status of the family can influence the outcome of a chronic illness.

- A chronic disease is a stressor to which children and families must adapt.
- The anxiety often leads to a maladaptive pattern of parent-child interactions and child behavior problems called the "vulnerable child syndrome".
- Chronic diseases constitute a major cause of mortality

- School presents particular challenges both physically and socially.
- The physical environment may contribute to exacerbation of chronic condition (dust, pollution, unfilled gas) and higher absenteeism.
- The social environment may include verbal abuse, less peer support, teacher's insufficient knowledge for support, leading to lower academic achievement in chronic ill children.

## Method and material

- The aim of this study was to evaluate the psychological characteristics of children and adolescents with different chronic illness.
- The examined groups comprise:

a) adolescents with **cystic fibrosis** (N = 25 mean age = 17.5 ± 2.6 years);

b) with **malignancies** (N = 20, mean age = 19.5 ± 1.3 years)

c) children with **juvenile rheumatoid arthritis** (N = 15, mean age 8.5 ± 0.56 years)

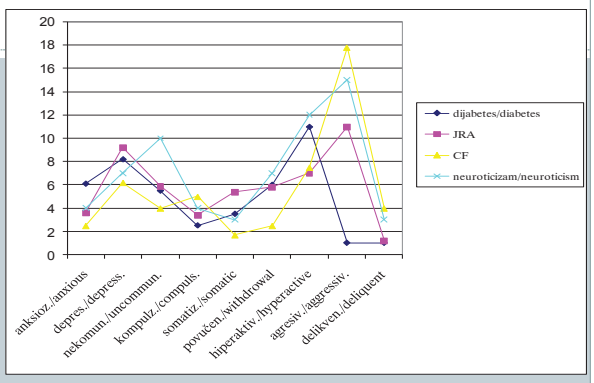
d) children with **diabetes mellitus** (N = 19, mean age = 12.5 ± 1.5 years)

We applied :

- Interview for all patients
- Child Behavior Checklist (CBCL) for children below 12 years,
- General Anxiety Scale (GAS),
- Eysenck Personality Questionnaire (EPQ) for children over 10 years,
- For adolescents and parents Minnesota Multiphase Personality Inventory (MMPI-201),
- Emotional Profile Index (PIE)
- Beck Depression Inventory (BDI) and
- Human Value Rank (HVR)

- The results obtained for EPQ and CBCL are compared with **control group** (25 healthy children, mean age 12.5 ± 0.98 years) as well as with patients diagnosed as **neuroticism** (N= 25, mean age 12.8 ± 1.5 years)

**CBCL**




## CBCL

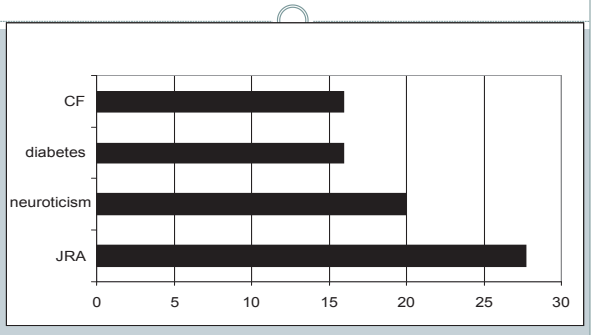
Three aspects of behavioral problems are more expressed:

- aggression,
- moderate depression and
- hyperactivity, but still within normal T-scores (below 65 percentile).

**EPQ obtained for chronic ill children**

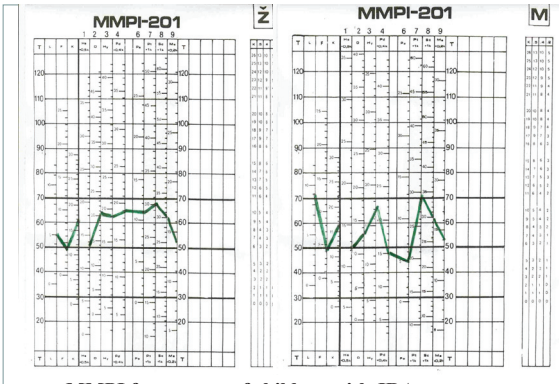
| *Condition* | *P* | *E* | *N* | *L* |
|---|---|---|---|---|
| Control | 11.87±6.23 | 13.16±5.75 | 13.84±5.31 | 12.64±4.62 |
| CF | 6.67±2.9 | 15.91±2.54 | 11.67±4.23 | 15±3.81 |
| JRA | 9.6±2.8 | 11.9±3.3 | 10.8±3.1* | 14.5±3.9 |
| Diabetes | 7.5±1.5** | 15.5±4.5 | 8±2** | 12±5 |
| Neuroticism | 5.16±3.1** | 14.16±1.7 | 16±3.6 | 12.6±4.1 |

**Results for General Anxiety Scale**




## GAS

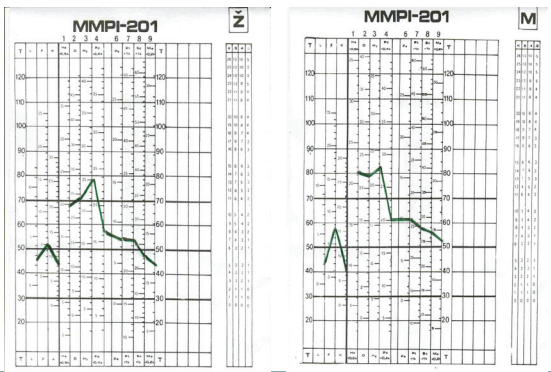
- Children with JRA are more anxious than other, except of neurotic patients
- The high anxiety can be related to the pain in JRA children and the reduction of movements in everyday life.




**MMPI for parents of children with JRA**

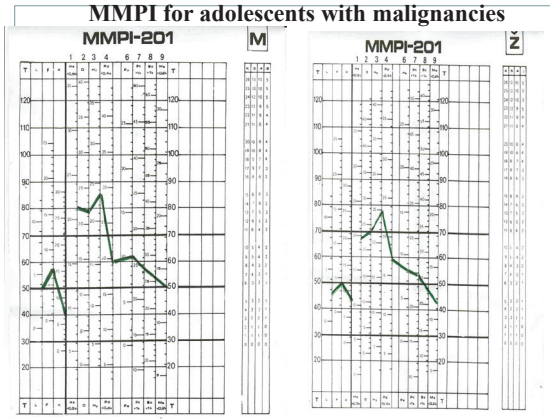
Fathers L-Hy-Pt profile, where Pt scores are higher than Hy score, which corresponds to accentuate psychopathic traits. For mothers the obtained profile is under T-scores but the Pt score is also the highest one.

**MMPI for adolescents with CF**




"neurotic" profile (Hs-D-Hy), especially in girls.

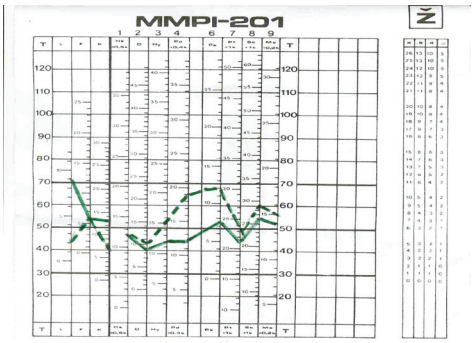
**MMPI for adolescents with malignancies**




Hs-D-Hy profile, which corresponds to hypersensitivity and high neurotic tendencies, combined with depression.

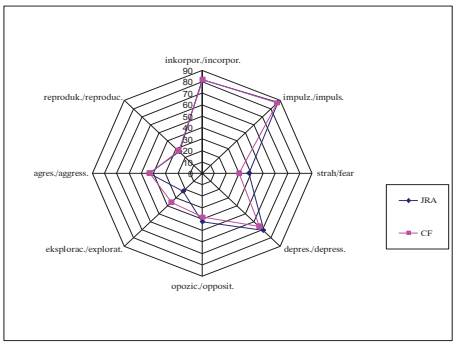
- The Beck depression inventory (**BDI**) was applied for cancer patients.
- The group's results showed total scores 14.66, which are under cutoff for depression.
- However, in 8 patients the score on BDI was over 19 which correspond to manifest depression

**MMPI profiles obtained for mothers and diabetic girls**




Pd-Pa-Sc correspond to the emotional instability as well as the possible psychopathic traits.

**EPI for CF and JRA adolescents**




Accentuated impulsivity and depression. The fear is most accentuated in JRA adolescents in opposite to exploration.


Human Value Rank for CF and diabetic children

## Conclusions

- The impact of a chronic disease on children and families is related less to a specific diagnosis than to disability profile and family functioning.
- Prognosis, predictability, the threat to life or to cognitive, social and physical development, the fears from medical and surgical interventions and the functional limitations have a major impact.
- One positive approach to chronic illness is to consider the factors that enable most children and families to cope as well as they can.

- Warning signs of distress in children include **problems at school** or in **social relationships**, **low self-esteem, helplessness/ hopeless** and **denial**, as well as a **poor compliance with treatment**.
- Generally, children and adolescents with chronic illness in our study appeared to be well adjusted with the diseases and did not manifested big mental problems.
- Most frequent psychological problems appeared to be **anxiety, sub-depression, oppositional behavior and aggressiveness.**

- Multidisciplinary teamwork can improve the care, quality of life and prognosis for both children and their families.
- Interventions, which are beneficial, include family therapy, supportive counseling or biofeedback modalities.
- Our experience with peripheral and central biofeedback treatment is very encouraging.

**LJILJANA SARANAC**

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## GROWTH HORMONE DEFICIENCY IN SOME RARE DISEASES

Growth failure is frequent characteristic in many rare diseases. However, growth hormone deficiency (GHD) is rarely documented in these patients.
We here report GHD in short stature girl diagnosed as Albright's hereditary osteodystrophy (pseudopseudohypoparathyroidism subtype) and in a boy with Aarskog syndrome. In a patient with Uveitis and suspected Lymphocytic hypophysitis, and in a boy with Non functioning pituitary microadenoma, short stature was also the principal cause for referral to endocrinologic examination. Partial or severe GHD was documented in all of them, and MRI (magnetic resonance imaging) revealed hypointense intrasellar lesions as a sign of disturbed tropic function. Growth hormone treatment was introduced with variable success.

Eventhough, growth impairment could be attributed or to be assumed as a part of complex clinical presentation of a particular rare disease *per se*, GHD should be suspected, documented and treated in these children.

# Growth hormone deficiency in some rare diseases

**Ljiljana Šaranac and Tatjana Stanković**

Pediatric Clinic, University of Niš
Serbia

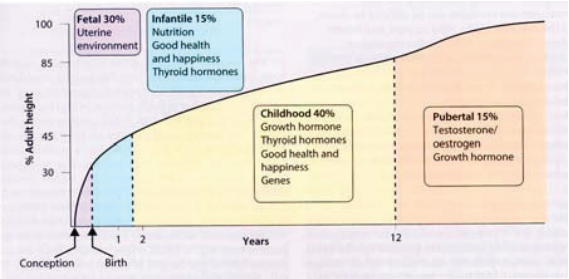
## Growth in 3 dimensions

Growth is not simple height augmentation, but also maturation.

The main regulator of postnatal growth is growth hormone (GH), influencing all 3 dimensions of growth.

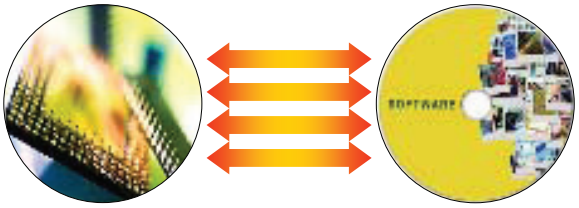
- Longitudinal growth
- Body composition
- Mineral bone content

## PHASES OF GROWTH IN CHILDHOOD




Lissauer T et Clayden G.Textbook of Paediatrics 2007.

## GHD CAUSES

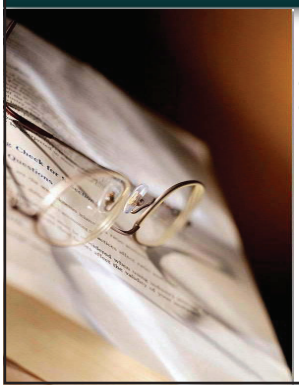



**Damaged hardware: genetic, head trauma**

**Wrong signals: nutrition, emotions**

**Damaged software (programming): SGA, Silver Russel, Prader Willi**

## HOW IDIOPATHIC IS IDIOPATHIC GHD?



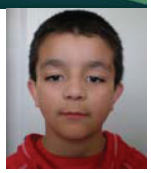
De Graff L. **Obstetric, neonatal, biochemical, immunologic, genetic and morphologic data of 244 Dutch GHD patients. Results of Hypopit study 2008.**

De Graff L, De Bellis A, Bellastella A, Hokken-Koelega. **Antipituitary antibodies in Dutch patients with idiopathic hypopituitarism**. *Horm Res* 2009; 71: 22-27

## SYNDROMA AARSKOG (FACIAL-GENITAL- DYSPLASIA : FGD)

- **Patient No 1, 9 years old boy**
- Normal BW/BL, 2 teeth present at birth
- Dysmorphic face
  (hypertelorism, antimongoloid eyes, short nasal bridge, anteverted nostrils, long philtrum, tiny lips, myopia)
- Short stature (proportional),

| | At diagnosis | 6 months GH th |
|---|---|---|
| Height (cm) | 118 (-2.4 SD) | 125.2 (-1.7SD) |
| HA (year) | 6.5 | 7.8 |
| BA (god) | 7.5 | / |
| BMkg | 31.5 (+9.5) | 33.5 (+8.5) |

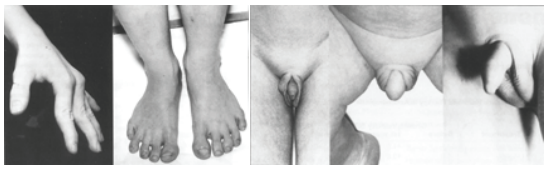


- **GH peak** (mU/l)

| | | |
|---|---|---|
| Clonidine test | 5.0 | |
| Insulin test | 12.5 | |
| IGF1ng/ml | 121 | 501 |
| IGFBP3 | 5.47 | |

## FGD (Sy Aarskog)

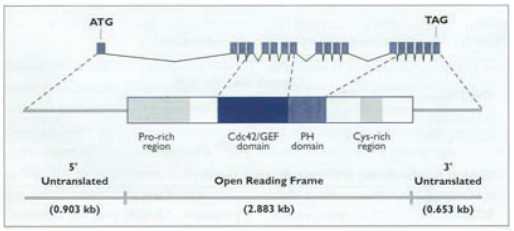
- The syndrome identified in 1967 by Dafginn Aarskog
- Principal features: short stature, facial, digital and genital malformations
- Short,broad hands with unique hyperextension of the PIP joint and extension of the distal joint
- Broad feet, bulbous toes
- Genital manifestations
  (cryptorchidism, inguinal hernias, very characteristic , "feminised skrotum"



Aarskog D. *Topical Endocrinology* 1998 (Suppl 3)

## INHERITANCE TYPE: RECESIVE, X linked

- FGD gene location: Xp11.21
- Activator of Cdc42 protein

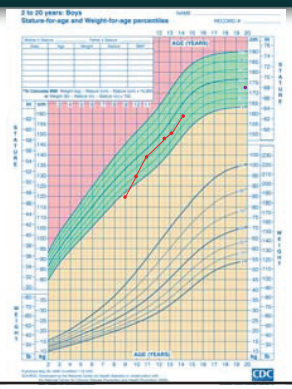



Genomic structure of Human FGDI

Aarskog D. *Topical Endocrinology* 1998 (Suppl 3)

## GROWTH CHART of SY AARSKOG

MPH
172.5



## GH STIMULATES GROWTH of SY AARSKOG

Petryk A, Richtan S, Blethen S. **The effect of GH tretment on stature in Aarskoog syndrome.** *J Pediatr Endocrinol Metab* 1999; 12: 161-5

Derendelier F, Larsson P, Neyezi O, Price A et al. **Growth hormone treatment in Aarskog syndrome: analysis of the KIGS.** *J Pediatr Endocrinol Metab* 2003; 16: 1137-42

## Patient No 2, Syndroma Albright

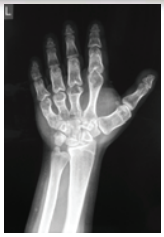
- **KM, 13.5 years old girl**
- SGA twin, BW 2050/BL 47,

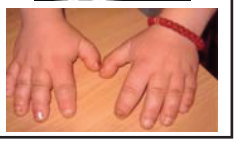
| | At diagnosis | At the end |
|---|---|---|
| • Height(cm) | 132 (-4 SD) | 132 (-4.8 SD) |
| • HA(years) | 8.75 | 8.75 |
| • BA (years) | 12.5 | / |
| • BM (kg) | 40.5 (+12.5) | 43(+15) |
| • Pub.stage | Tanner 1 | Tanner 3 |
| • **Growth (mU/l) peak** | | |
| Clonidine test | 11 | |
| Insulin test | 7.9 | |
| IGF1 (ng/ml) | 236 | 377 |
| TSH (mU/l) | 9.2 | 7.6 |
| Cortisol (nmol/l) | 667 | 707 |
| PTH (pg/ml) | 97 (n.8-76) | |

## Albrights Hereditary Osteodystrophy (AHO)

- Pseudopseudohypoparathyroidism PPHP type
- The syndrome described by Fuller Albright in 1942.
- Principal features: severe growth retardation, obesity
- Delayed puberty
- Dysmorphic round face, depressed nasal bridge
- Short neck
- Short stubby fingers, short metacarpals
- PTH elevated, normal calcium and phosphate level
- Multihormone resistance: PTH, TSH, LH, GHRH...

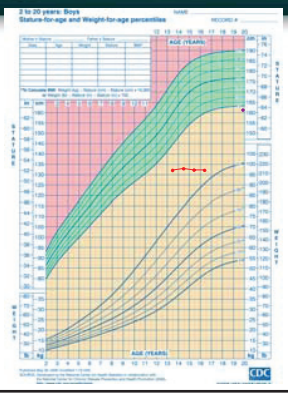
## Patient No 2 (AHO)



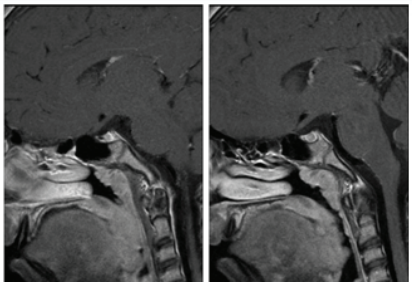


## Growth chart of patient No 2

MPH
165.5



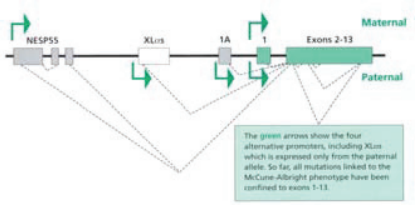
## Pituitary MRI



- Hypodense intrasellar mass
- Microadenoma

## Genetic of Sy Albright

- Partial deficiency of the alfa subunit of G protein (GsA), encoded by the GNAS gene
- Paternal imprinting




**The GNAS1 Gene and Maternal and Paternal promoters**

De Zegher F 2004

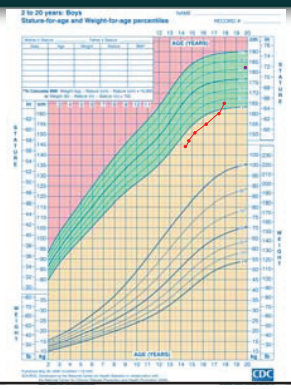
## Patient No 3, Uveitis

- **SN, 14.3 years old boy**
- Dg. Uveitis
  Treatment; Pronison 5 mg/daily + Sandimun 2X50 mg daily

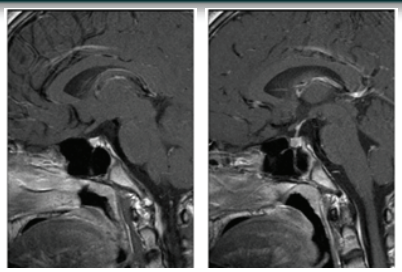
| | At diagnosis | I control (4 months GH) |
|---|---|---|
| • Height(cm) | 143.7 (-2.6 SD) | 146 (-2.3 SD) |
| • HA(years) | 11 | 11.5 |
| • BA (years) | 9.5 | / |
| • BM (kg) | 38.3 (+2.5) | 41 (+3) |
| • Pub.stage | Tanner 1 | Tanner 2 |
| • **Growth (mU/l) peak** | | |
| Clonidine test | 17 | |
| Insulin test | 3.9 | |
| IGF1 (ng/ml) | 270 | 660 |
| GnRH test: | prepubertal response | |
| Cortisol (nmol/l) | 217...229 | 306 |
| ACTH (ng/ml) | 47 | |

## Growth chart of pat No 3

MPH
182



## Pituitary MRI

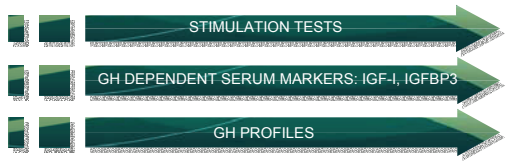


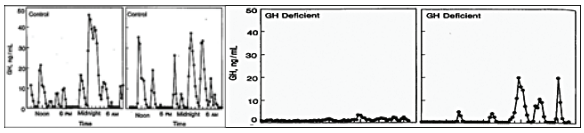
- Hypodense intrasellar mass
- Microadenoma
- Hypophysitis?

## What is common: pituitary lesions

- Functional investigation
- Imaging diagnostic
- Genetic?
- Pituitary autoantibodies?

## Biochemical Evaluation of GHD




## Confirmation of GHD

- Short stature or low GV
- Bone age retardation
- GH response in provocation test less than 10ng/ml (<20mIJ/l)
- Low IGF-1 and IGFBP-3 (< -2SD)

GH Research Society Consensus guidlines for the diagnosis and treatment of growth hormone (GH) deficiency in childhood and adolescence: Summary Statement of the GH Research Society. J Clin Endocrinol Metab 2000; 85: 3990-3993.

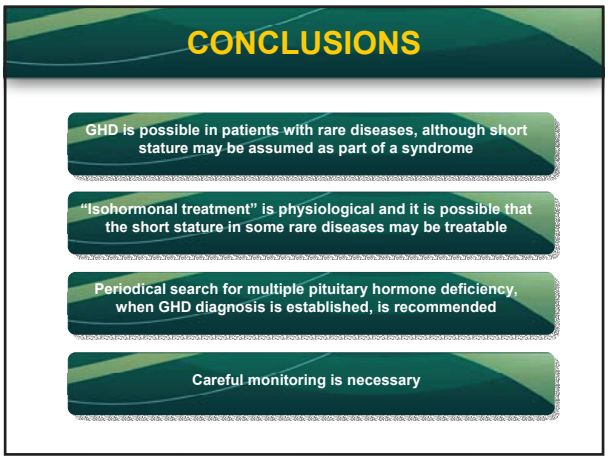
## Gandrud LM, Darrell MW.

**Is growth hormone stimulation testing in children still appropriate?**

*Growth Hormone & IGF Research* 2004; 14(3): 185-194.

## FALSE NEGATIVE or POSITIVE RESULTS

- **GHD types:**
  - **Severe**
  - **Definitive/Certain**
  - **Uncertain**
    - Seasonal
    - Cyclic variations
    - Transient


CONCLUSIONS
GHD is possible in patients with rare diseases, although short stature may be assumed as part of a syndrome
"Isohormonal treatment" is physiological and it is possible that the short stature in some rare diseases may be treatable
Periodical search for multiple pituitary hormone deficiency, when GHD diagnosis is established, is recommended
Careful monitoring is necessary

**ZORAN GUCEV**

*University Pediatric Clinic, Medical Faculty Skopje*

## RARE DISEASES, NEW MOLECULAR INSIGHTS GAINED

We present a series of patients with rare diseases with an emphasis on the molecular mechanisms learned. For the purpose of the abstract we present an overgrowth syndrome with the molecular lessons learned. Mutations in key genes of the phosphatidylinositol-3-kinase (PI3K)/AKT signaling pathway have been identified in numerous tumor samples, while loss of PTEN function or activation of AKT1, AKT2 or AKT3 have been implicated in disorders that feature overgrowth and/or hypoglycemia. An exome sequencing of DNA from affected and unaffected skin fibroblasts from a patient (C1) with unclassified severe overgrowth of the right leg identified a cancer-associated variant in PIK3CA in DNA from the affected sample that was not present in the unaffected sample (c.3140A>T which predicts p.His1047Leu). The alteration was also found in DNA isolated from other affected tissues from muscle, bone, fibrous and adipose tissue. These patients did not meet the clinical criteria for Proteus syndrome, matching those of CLOVES syndrome. However, these patients lacked the complex truncal vascular malformations that are commonly found in patients with CLOVES. Thus the spectrum of phenotypes associated with somatic activation of PI3K signaling is expanded and multiple therapeutic targets are suggested.

# Rare diseases: new molecular insights

Zoran S. Gucev,[1] Natasa Cuckova[1], Velibor B. Tasic[1]
[1]Medical Faculty Skopje, Macedonia

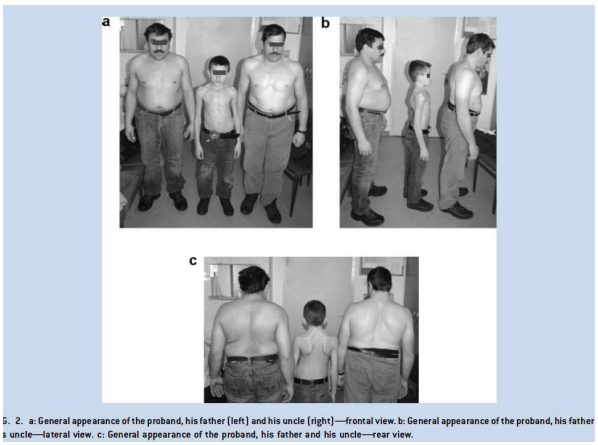



FIG. 2. a: General appearance of the proband, his father [left] and his uncle [right]—frontal view. b: General appearance of the proband, his father and his uncle—lateral view. c: General appearance of the proband, his father and his uncle—rear view.

CLINICAL REPORT

AMERICAN JOURNAL OF medical genetics PART A

## Autosomal Dominant Spondylocostal Dysostosis in Three Generations of a Macedonian Family: Negative Mutation Analysis of *DLL3*, *MESP2*, *HES7*, and *LFNG*

Zoran S. Gucev,[1]* Velibor Tasic,[1] Nada Pop-Jordanova,[1] Duncan B. Sparrow,[2,3] Sally L. Dunwoodie,[2,3] Sian Ellard,[4] Elizabeth Young,[4] and Peter D. Turnpenny[4,5]



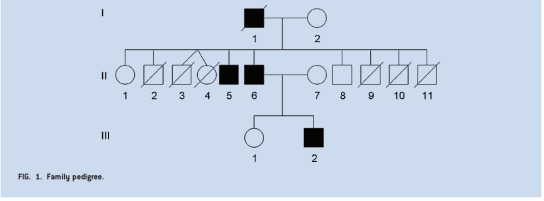
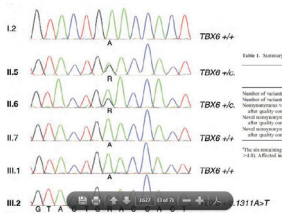



FIG. 1. Family pedigree.

Human Molecular Genetics, 2013, Vol. 22, No. 8 1625–1631
doi:10.1093/hmg/ddt012
Advance Access published on January 17, 2013

## Autosomal dominant spondylocostal dysostosis is caused by mutation in *TBX6*

Duncan B. Sparrow[1,2,†], Aideen McInerney-Leo[4,†], Zoran S. Gucev[5], Brooke Gardiner[4], Mhairi Marshall[4], Paul J. Leo[4], Deborah L. Chapman[6], Velibor Tasic[5], Abduhadi Shishko[7], Matthew A. Brown[4,†], Emma L. Duncan[4,8,†] and Sally L. Dunwoodie[1,2,3,*,†]



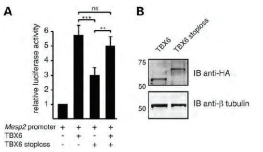




Figure 3. Functional analysis of the TBX6 mutation. (A) Mutant TBX6 activates transcription from a *Mesp2* promoter statistically significantly less effectively than the wild-type protein. ***$P < 0.001$; **$P < 0.01$; ns, not significant. An equimolar mixture of wild-type and mutant TBX6 activates transcription at an intermediate level. Assays were performed in triplicate. Error bars represent standard deviations of four independent experiments. One-way analysis of variance was performed on data from all four experiments, and significance was determined using Tukey's *post hoc* test. (B) The p.*437Cext*81 mutation does not adversely affect TBX6 protein translation efficiency or stability. Western blot of protein extracts from C2C12 mouse muscle satellite cells transiently transfected with expression constructs encoding HA-tagged wild-type and mutant TBX6.

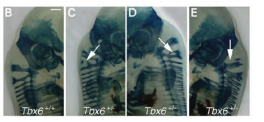
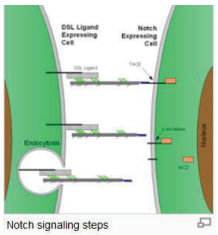
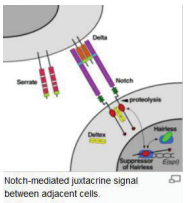



Figure 4. Heterozygous null *Tbx6* mouse embryos have mild vertebral defects with variable penetrance. (A) Histograms showing the incidence of abnormalities observed in the cervical and sacral regions of E14.5 embryos stained with alcian blue. The total number of embryos of each genotype is shown below each histogram. (B–E) Lateral views of alcian blue-stained E14.5 embryos showing typical examples of the observed cervical vertebral defects. Arrows indicate the location of the defects. (B) The distal part of the spinous process is not attached to the rest of the spinous process. (C) A foramen is present in the most distal portion of the spinous process. (D) The distal part of the spinous process is not attached to the rest of the spinous process.

# Notch Signaling Pathway



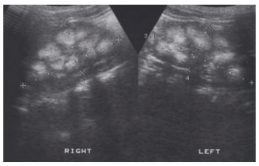




*Fig. 1.* Bilateral medullary hyperechogenicity mimicking nephrocalcinosis.

Table 1. Plasma and urine concentration of purine metabolites before and after treatment with allopurinol (5 mg/kg)

| | Pre-allopurinol (normal range) | Post-allopurinol (normal range) |
|---|---|---|
| Uric acid (p) | 0.760 (0.100–0.260) | 0.343 (0.100–0.260) |
| Xanthine (p) | ND | 0 (<1) |
| Hypoxanthine (p) | ND | 0.670[a] (<1) |
| Oxypurinol (p) | – | 0.026 |
| Uric acid (u) | 3.716 | 2.960 |
| Xanthine (u) | 0.044 | 2.635 |
| Hypoxanthine (u) | 0.132 | 2.785 |
| Creatinine (u) | 1.0 | 2.6 |
| Ratio uric acid/ creatinine (u) | 3.7 (<1.5) | 1.1 (<1.5) |
| Ratio total oxypurines/ creatinine (u) | 3.9 (<1.5) | 3.2 (<1.5) |
| Oxypurinol (u) | – | 0.417 |

...done; u, urine.
...he elevated due to time sample spent in transit. All values are expressed as mmol/l.

## Gucev Z, Tasic V et al. Clin Genet 209:796-798.

Normal TCCTCATGGA CTAATTATGG ACAGgtaag- -------taa gatct -intron 3

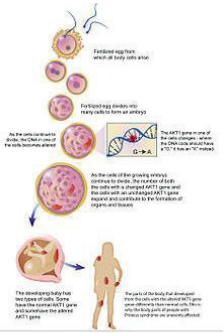
Variant TCCTCATGGA CTAATTATGG*ACTAATTATG*GACTAATTAA GATCT

*Fig. 2.* A comparison between the normal and variant hypoxanthine guanine phosphoribosyltransferase 1 (HPRT1) exon 2 genomic sequences. The authentic intron 3 splice donor sequence is shown boxed in lower case. The sequence motif *GACTAATTATG* is duplicated in the sequence variant, the intron 3 splice donor site is lost, and an additional 8 bases inserted.

- In Greek mythology, **Proteus** (Πρωτεύς) is an early sea-god, one of several deities whom **Homer** calls the "Old Man of the Sea".[1]
- the god of **"elusive sea change,"** which suggests the constantly changing nature of the sea
- The adjective **protean**, with the general meaning of **"versatile", "mutable", "capable of assuming many forms"**.

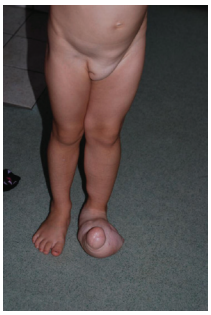
## PROTEUS OR WIEDEMAN SYNDROME (AKT1 gene)

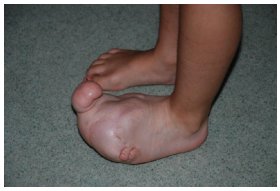
Proteus as envisioned by Andrea Alciato



Proteus syndrome is an overgrowth disorder caused by a rare genetic mosaicism. A genetic mutation during embryonic develop gives rise to overgrowth in a subset of the individual's cells

## A 4.5 year old girl



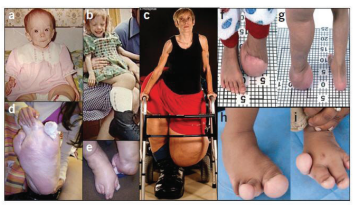


## Differentials

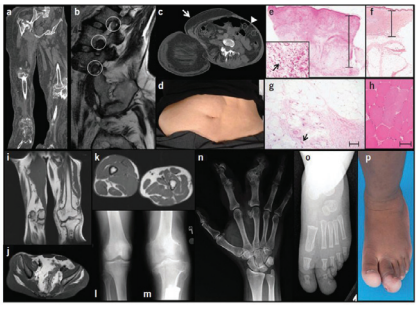
- 1. Proteus syndrome: lacks cerrebriform connective tissue naevus,
- 2. Klippel-Trnaynay syndrome: no lateral venous anomaly and port vine stain

Lindhurs M… Gucev Z…et al. **Nature Genetics** 2012;44(8):928-33.

**Figure 1** Spectrum of overgrowth in individuals with activating *PIK3CA* mutations. (a–e) Subject C1, showing lower-extremity overgrowth with a paucity of facial adipose tissue at ages 12 months (a) and 7 years (b) progressing to massive leg overgrowth with lack of upper-body adipose tissue at 37 years, by which time above-knee amputation of the left leg had been undertaken (c). (d) Right foot overgrowth at 35 years old. (e) Feet at 35 years, showing overgrowth, cutaneous syndactyly of the left foot and rotational deformity of the right foot. (f,g) Anterior (f) and posterior (g) views of subject N7, showing leg status after debulking surgery and massive overgrowth of the left foot, which was partially amputated. (h,i) Right (h) and left (i) feet of subject N99, showing more limited overgrowth confined to several rays of the feet. Informed consent to publish photographs was obtained from the identifiable individual, subject C1.



Lindhurs M… Gucev Z…et al. **Nature Genetics** 2012;44(8):928-33.



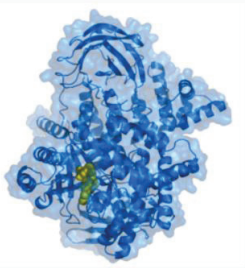
Lindhurs M… Gucev Z…et al. **Nature Genetics** 2012;44(8):928-33.

**Table 1 Summary of clinical features of affected individuals**

| Subject ID | C1 | C2 | N7 | N45 | N68 | N99 | N104 | N108 | N109 | N110 |
|---|---|---|---|---|---|---|---|---|---|---|
| Age in 2012 (years) | 37 | 31 | 17 | 31 | 49 | 1 | 11 | 6 | 4 | 1.5 |
| Sporadic occurrence[a] | + | + | + | + | + | + | + | + | + | + |
| Mosaic distribution[a] | + | + | + | + | + | + | + | + | + | + |
| Progressive course[a] | + | + | + | + | + | +/– | + | + | + | +/– |
| Linear epidermal nevus[a] | – | – | – | +/– | – | – | – | +/– | – | – |
| Asymmetry, disproportionate limb overgrowth[a] | + | + | + | + | + | + | + | + | + | + |
| Fibroadipose overgrowth[a] | – | – | + | + | + | + | + | + | + | |
| Regional lipohypoplasia[a] | + | – | + | + | – | – | | + | – | – |
| Vascular malformations, one or more[a] | – | – | – | + | + | – | – | – | – | – |
| Polydactyly | – | – | + | – | – | – | – | – | – | – |
| Non-spleen/thymus visceral overgrowth | – | – | – | + | – | | | – | – | – |
| Lipomatous infiltration of muscles | + | + | + | + | + | | | + | | |
| Testicular or epididymal abnormalities | – | – | + | + | + | | | | | |
| Natural history features | | | | | | | | | | |
| Congenital overgrowth | + | + | + | + | + | + | | + | | – |
| Progression of adipose dysregulation | + | + | + | + | + | | | + | | |

+/–, some evidence to support presence of feature; empty cell, not assessed.
[a]Diagnostic feature of Proteus syndrome. Other specific criteria for Proteus syndrome that were either absent or not assessed in all subjects are not enumerated.

**Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha**



PI3 Kinase 110 alpha bound to the inhibitor PIK-93 (yellow).

Lindhurs M… Gucev Z…et al. **Nature Genetics** 2012;44(8):928-33.

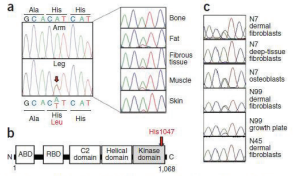



**Figure 3** Identification of *PIK3CA* mutations in affected cells and tissues. (a) The *PIK3CA* c.3140A>T mutation (p.His1047Leu) identified in cultured dermal fibroblasts from the affected left leg but not the unaffected right arm of subject C1 (left) and present at varying levels in left leg tissues (right). (b) Location of His1047 near the C terminus of the kinase domain of the p110α catalytic subunit of type IA PI3K. ABD, adaptor-binding domain; RBD, RAS-binding domain. (c) The *PIK3CA* c.3140A>G mutation (p.His1047Arg) identified in cells derived from a variety of tissues from subjects N7, N99 and N45.

Lindhurs M… Gucev Z…et al. **Nature Genetics** 2012;44(8):928-33.

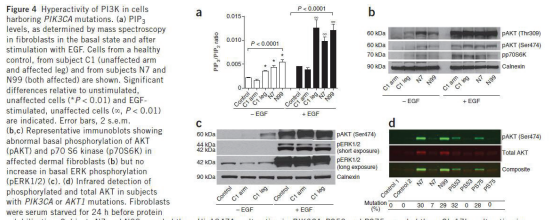


**Figure 4** Hyperactivity of PI3K in cells harboring *PIK3CA* mutations. (a) $PIP_3$ levels, as determined by mass spectroscopy in fibroblasts in the basal state and after stimulation with EGF. Cells from a healthy control, from subject C1 (unaffected arm and affected leg) and from subjects N7 and N99 (both affected) are shown. Significant differences relative to unstimulated, unaffected cells (*$P < 0.01$) and EGF-stimulated, unaffected cells (∞, $P < 0.01$) are indicated. Error bars, 2 s.e.m. (b,c) Representative immunoblots showing abnormal basal phosphorylation of AKT (pAKT) and p70 S6 kinase (p70S6K) in affected dermal fibroblasts (b) but no increase in basal ERK phosphorylation (pERK1/2) (c). (d) Infrared detection of phosphorylated and total AKT in subjects with *PIK3CA* or *AKT1* mutations. Fibroblasts were serum starved for 24 h before protein solubilization. Subjects N7 and N99 encoded the p.His1047Arg alteration in *PIK3CA*, PS53 and PS75 encoded the p.Glu17Lys alteration in AKT1 (ref. 3), and Control 1 and 2 were from unaffected individuals and were negative for both alterations.

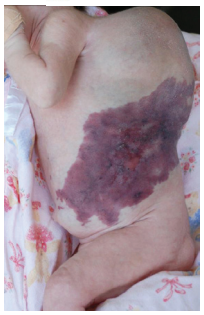
## Also found in >130 cancers:

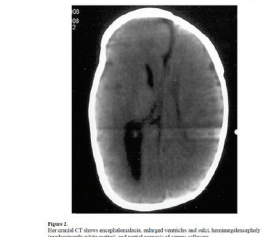
- BREAST CANCER, SOMATIC
- OVARIAN CANCER, EPITHELIAL, SOMATIC, INCLUDED
- COLORECTAL CANCER, SOMATIC, INCLUDED
- GASTRIC CANCER, SOMATIC, INCLUDED
- HEPATOCELLULAR CARCINOMA, SOMATIC, INCLUDED
- NONSMALL CELL LUNG CANCER, SOMATIC, INCLUDED

*Am J Med Genet A*. 2008 October 15; 146A(20): 2688–2690. doi:10.1002/ajmg.a.32515.

**CLOVE Syndrome (Congenital Lipomatous Overgrowth, Vascular Malformations, and Epidermal Nevi): CNS Malformations and Seizures may be a Component of this Disorder**

Zoran S. Gucev[1], Velibor Tasic[1], Aleksandra Jancevska[1], Marina Krstevska Konstantinova[1], Nada Pop-Jordanova[1], Zoran Trajkovski[1], and Leslie G. Biesecker[2]
[1] Medical Faculty Skopje, 50 Divizija BB, 1000 Skopje, Macedonia
[2] National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, USA





**Kurek, K. C. Somatic mosaic activating mutations in PIK3CA cause CLOVES syndrome. *Am. J. Hum. Genet.* 90: 1108-1115, 2012.**

**Foukas et al.** Critical role for the **p110-alpha phosphoinositide-3-OH kinase** in growth and metabolic regulation. **Nature** 441: 366-370, 2006.

- **Mice heterozygous for this mutation displayed severely blunted signaling via insulin-receptor substrate (IRS) proteins (e.g., 147545), key mediators of insulin, insulin-like growth factor-1 (IGF1; 147440), and leptin (164160) action. Defective responsiveness to these hormones led to reduced somatic growth, *hyperinsulinemia, glucose intolerance, hyperphagia, increased adiposity* risk in mice heterozygous for the D933A mutation.**

## SNEZANA BOJCIN

# CYSTIC FIBROSIS IN MACEDONIA

Enhancing quality of life and living a longer and better life

## INTRODUCTION

SNEZANA BOJCIN

- mother of a CF child
- founder and president of MCFA
- CFE board member

## WHAT IS CYSTIC FIBROSIS

According to ORPHANET and EURORDIS Cystic Fibrosis or mucoviscidose is the most common life threatening inherited disease in Europe.

Sticky mucus blocks the respiratory and digestive system.
CF is an incurable disease.

Early diagnosis, regular follow-up by a multidisciplinary team in a specialized CF clinic, proper hygiene and correct, timely treatment of symptoms can prolong and save lives and improve the quality of life.

The disease is chronic and generally progressive, with onset usually occurring during early childhood.

The most common form of CF is associated with respiratory symptoms, digestive problems and staturoponderal growth anomalies.

## CF in Europe

CF affects approximately one in every 2500 children born.

Nearly 50.000 people are living with CF in Europe, but this is probably an underestimate due to lower access to diagnosis and care in newer EU countries and eastern Europe.

CF is ranked as one of the most widespread life-shortening genetic diseases.

According Orhanet in the 1960's the majority of patients died before 5 years of age, whereas the current average life-span exceeds 35 years and life-expectancy is 40 years in EU countries.

## CF in Macedonia

- CF is still not officially recognized as a rare disease
- 128 CF patients are registered at this moment
- Expected number of patients according EU statistics should be 200-250
- The oldest registered CF patient in Macedonia is 30 years old.
- Only about 20% of CF population are adults.
- Comparing 67 patients in year 2000 we have doubled a number of patients today.

## FUTURE PLANS

The treatment needs a number of different drugs which are still not available in our country:

- Antibiotics,
- Antifungal drugs,
- Multivitamin supplements,
- Hippercaloric supplementary food

We also need:

- CF center for CF Adult patients,
- Physiotherapy devices and devices for regular inhalation therapy which are very expensive,
- CF transplantation program.

**Which means implemented ECFS Standards of care**

## H. JOACHIM SEITZ

*Dir. (em) Institute Biochemistry and Mol. Biology III: Mol. Endocrinology,*
*University Medical Center Hamburg-Eppendorf, Germany*

### THYROID HORMONE RESISTANCE – MOLECULAR BASIS, CLINICAL PICTURE

Thyroid hormones play an essential role in the development of brain, heart, skeletal muscle and other organs in utero and after birth. Thyroid hormones exert their action via the nuclear receptor alpha and beta. Both are members of the steroid/retinoic acid/vit. D receptor super family of ligand-inducible transcriptions factors. When DNA sequencing was routinely available, it was possible to elucidate thyroid hormone action at the nuclear level and to discover rare disorders of thyroid hormone resistance due to mutations in the alpha or beta receptor.

In the talk an overview on the following topics will be presented: the steroid/retinoic acid/ vit. D/T3 nuclear receptor family, molecular structure, hormone binding, transcription activation, hormone responsive elements, principles of hormone resistance, and T3 receptor defects and accompanying clinical features.

Universitätsklinikum Hamburg-Eppendorf

Skopje Nov 15/16 2013: RARE DISEASES

## THYROID HORMONE RESISTANCE

**Prof. Dr. med. H. Joachim SEITZ**

**Director (pens.) Institute Biochemistry & Mol Biologie III: Endocrinology - UKE**

## TR-Alpha Resistance

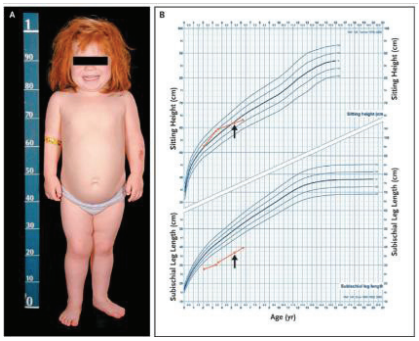
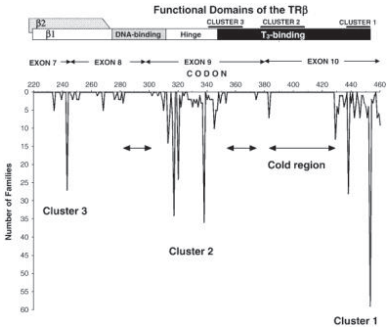


**Figure 1. Phenotypic Features of the Patient.**

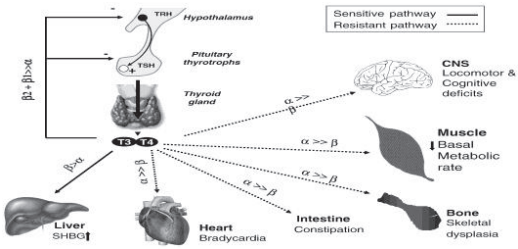
A photograph of the patient (Panel A) illustrates relative macrocephaly and skeletal disproportion. The chart in Panel B indicates linear growth retardation (in red), which affected the lower segment of her body, with arrows denoting the initiation of thyroxine treatment

Citation: K. Chatterjee et al. A Mutation in the Thyroid Hormone Receptor Alpha Gene, NEJM January 2012; 366:243-249

## Location of natural mutation in the TRβ molecule associated with RTH



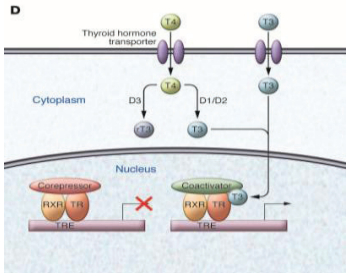

Citation: AM Dumitrescu et S Refetoff, The Syndromes of Reduced Sensitivity to Thyroid Hormone, Biochimica et Biophysica Acta (BBA), Vol 1830, 2013, 3987-4003




Major pathways of thyroid hormone action are shown, together with the principal receptor subtypes (TRα or TRβ) mediating such effects in different target tissues.

Citation: N Schoenmakers et al., Resistance to thyroid hormone mediated by defective thyroid hormone receptor alpha, Biochimica et Biophysica Acta (BBA), Vol 1830 (7) 2013, 4004 – 4008

## Nuclear Action of Thyroid Hormone




Citation: GA Brent, Mechanisms of Thyriod Hormone Action, JCI, 122, 3035-3043, 2012

Abstract Meeting in Skopje, Nov 16./17. 2013

Prof. Dr. med. H. Joachim Seitz,
Dir. (pens) Institute Biochemistry and Mol. Biology III: Mol. Endocrinology, University Medical Center Hamburg-Eppendorf, Germany.

**Thyroid Hormone Resistance**

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In the talk an overview on the following topics will be presented: the steroid/retinoic acid/vit. D/T3 nuclear receptor family, molecular structure, hormone binding, transcription activation, hormone responsive elements, principles of hormone resistance, and T3 receptor defects and accompanying clinical features.

Review paper:


- The syndromes of reduced sensitivity to thyroid hormone. A. M. Dumitrescu & S. Refetoff. BBA 1830, 3987 (2013)
- Thyroid hormone transporter and resistance. T. J. Visser. In: Hormone Resistance and Hypersensitivity. vol 24, p 1 – 10 (2013) (doi:10.1159/000343695)
- Resistance to thyroid hormone mediated by defective thyroid hormone receptor alpha. K. Chatterjee et al. BBA 1830, 4004 (2013)
- A mutation in the thyroid hormone receptor alpha gene. K. Chatterjee et al. NEJMed 366, 243 (2012)
- Mechanisms of thyroid hormone action. G. A. Brent. J Clin Invest122, 3035 (2012)
- Coordination of mitochondrial biogenesis by thyroid hormone. J. M. Weitzel & K. A. Iwen. Mol Cell Endocrinol 342, 1 (2011)

**VUKASIN ANDRIC**
Genzyme, Zagreb, Croatia

## ENZYME REPLACEMENT THERAPY IN GAUCHER DISEASE

Gaucher disease is a autosomal recessive inherited lysosomal storage disorder (LSD) caused by a deficiency of certain lysosomal enzymes necessary to break down carbohydrates. Carbohydrates progressively accumulate in the lysosomes of the body's cells. The result is significant cellular, tissue, and organ dysfunction. The glucocerebrosidase gene has been mapped to chromosome 1q21. There are over 150 identified glucocerebrosidase mutations. They causes a deficiency of acid glucocerebrosidase, an enzyme that helps to break down glucosylceramide. "Gaucher cells" are macrophages where glucosylceramide is stored, commonly found in the bone marrow, spleen, and liver of patients with GD. The definitive diagnosis of Gaucher disease is based on a deficiency of glucocerebrosidase enzyme activity. However, the level of residual enzyme activity does not enable prediction of clinical outcome or disease severity. DNA analysis provides additional confirmation of the clinical diagnosis. Certain mutations in affected patients are associated with mild or severe disease. Thus, management decisions need to be individualized. Recombinant DNA technology is used for the process of manufacturing Enzyme replacement therapy (ERT). A plasmid is taken from a bacterium and a copy of the human gene for the enzyme glucocerebrosidase is inserted into that plasmid. It is then inserted into the nucleus of a host cell where it causes the host cell to produce the target protein. The target protein can then be harvested from the culture medium, collected and purified into the therapeutic enzyme. Infused ERT enters a macrophage (Gaucher cell) and breaks down glucosylceramide that has accumulated in the lysosome.

# Enzyme Replacement Therapy for Gaucher Disease

**Rare Disease in SEE meeting, Skopje, Macedonia, November 16, 2013**

***Dr.Vukasin Andric, Genzyme***

1

## Gaucher Disease is a *Lysosomal Storage Disorder* (LSD)

The LSDs are a family of genetic diseases resulting in a pathologic build-up of undegraded storage material in lysosomes

Collectively, LSDs occur in 1 / 6,000 - 7,000 live births

All LSDs are multisystemic, chronic and progressive

More than 40 LSDs have been identified

A subset of LSDs → Gaucher disease (3 clinical sub-types)

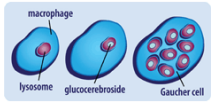
~95% of Gaucher cases → Gaucher disease type 1 (GD1)

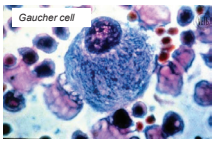
Grabowski GA, Lancet 2008;372:1263.



## Lysosomal Storage Disorder (LSD)

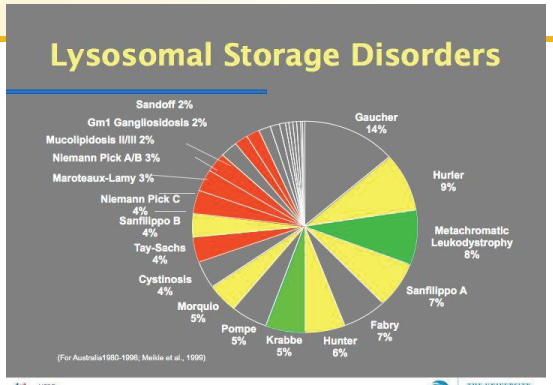
- All LSDs are caused by a deficiency of certain lysosomal enzymes necessary to break down carbohydrates.
- The pathology differs depending on the deficient enzyme.
- In all LSDs, carbohydrates that are broken down partially or not at all progressively accumulate in the lysosomes of the body's cells.
- The result is significant cellular, tissue, and organ dysfunction.






## Lysosomal Storage Disorders

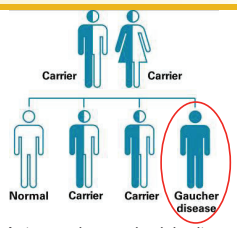



NFRD Program for Neurodevelopmental Function in Rare Disorders University of North Carolina - Chapel Hill

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL



## Inheritance and Epidemiology




- Autosomal recessive inheritance
  - Both parents must be carriers of GD
  - 25% chance any child of these parents will have GD
- Overall population prevalence of GD1 is ~1:100,000
  - GD is pan-ethnic
  - Most common in Ashkenazi Jewish population (1:450-1:2,500)
- Most common mutations in the *GBA* gene (~95% of all cases): N370S, L444P, 84GG, IVS2
- Phenotype-genotype correlations:
  - GD1 varies widely in clinical expression, and patients present with a broad spectrum of phenotypes
  - Patients may have severe disease in childhood or may remain essentially symptom-free into late adulthood

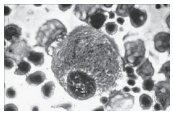
Grabowski GA, Lancet 2008;372:1263.



## Pathogenesis of Gaucher Disease
### Progressive, Multisystemic, Multiorgan Dysfunction

- Substrate-engorged macrophages, called "Gaucher cells," accumulate in the affected organs, leading to the following common symptoms:
  - Visceral enlargement
    - Splenomegaly (5-75x normal)
    - Hepatomegaly (2-3x normal)
  - Hematological abnormalities
    - Thrombocytopenia
    - Anemia
  - Bone disease
    - Bone pain & bone crisis
    - Osteoporosis
    - Pathologic fractures, joint collapse
    - Osteonecrosis
- Other organs can be affected.
  - Lung (pulmonary failure)
- Even mild disease can significantly decrease quality of life.

Bone Marrow



*Gaucher cell*

Beutler E, Grabowski GA, in The Online Metabolic & Molecular Bases of Inherited Disease, Scriver et al., eds. (The McGraw-Hill Companies, New York, NY, 2006), pp. 3635-3668.



## Gaucher Disease Phenotypes

| | Type 1 non-neuronopathic | Type 2 acute neuronopathic | Type 3 chronic neuronopathic |
|---|---|---|---|
| Prevalence | 1/50,000 (pan-ethnic) 1/500 (Ashkenazi Jews) | 1/100,000 (pan-ethnic) | 1/100,000 (pan-ethnic) |
| Age at presentation | Any | Infancy | Childhood |
| Lifespan | 6 to > 80 y | ~ 2 y | 2 to 60 y |
| Primary CNS disease | none | severe | mild to severe |
| Visceromegaly | mild to severe | moderate to severe | mild to severe |
| Hematologic abnormalities | mild to severe | severe | mild to severe |
| Skeletal abnormalities | none to severe | none | none to severe |

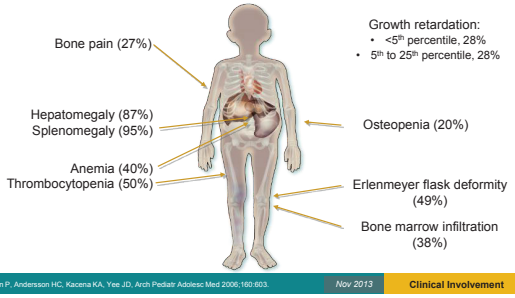
**Distinguishing between types 1 and 3 in children can be difficult, as CNS symptoms may be subtle in younger patients.**

Meikle PJ et al, JAMA 1999;281:249. Poorthuis BJet al. Hum Genet 1999;105:151.



## GD1 Presentation in Children

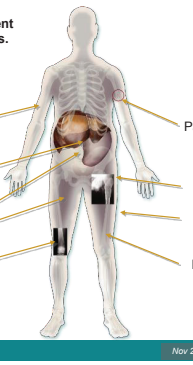
**Children or adolescents with GD1 often have marked splenomegaly, easy bruising/bleeding/hypermenorrhagia and slower than normal growth and pubertal development.**




Kaplan P, Andersson HC, Kacena KA, Yee JD. Arch Pediatr Adolesc Med 2006;160:603.

## GD1 Clinical Presentation
### A Multisystemic Disorder

**Patients with GD1 can present with any of these symptoms. Some may be severe and others completely absent.**



Bone pain (63%)
Bone crisis (33%)
Hepatomegaly (79%)
Splenomegaly (87%)
Anemia (64%)
Thrombocytopenia (56%)
Erlenmeyer flask deformity (46%)

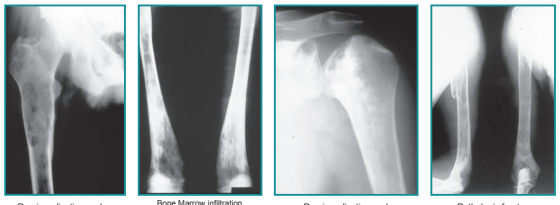
General symptoms:
- Fatigue
- Easy bruising/bleeding
- Menorraghia
- Decreased appetite
- Abdominal pain

Pathologic fracture (15%)
Joint collapse (8%)
Osteonecrosis (25%)
Osteopenia (42%)
Bone marrow infiltration (40%)



## Bone Pathology May Be Irreversible

- Gaucher disease-related manifestations are progressive
- Skeletal tissue necrosis is irreversible and may lead to joint replacement
- Demineralisation can lead to osteopenia/osteoporosis and pathological fractures
- Prevention is key to avoid serious consequences of bone pathology



Demineralization and osteonecrosis - Femur | Bone Marrow infiltration and Erlenmeyer flask deformity - Femur | Demineralization and osteonecrosis - Humerus | Pathologic fracture Humerus



## Additional Symptoms and Co-morbidities Associated with GD1

- Neoplastic disorders
  - Chronic lymphocytic leukemia, multiple myeloma, Hodgkin and non-Hodgkin lymphoma
- Parkinson disease
- Pulmonary hypertension
- Hypermetabolic / underweight
- Inflammatory markers



## Gaucher Disease Type 1 Shortens Life Expectancy

Brief Research Report

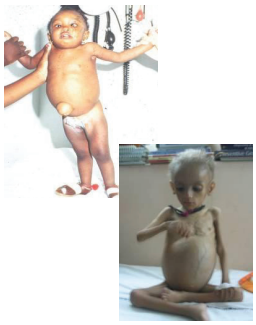
Life expectancy in Gaucher disease type 1

- Perception
  - GD1 affects quality of life, but not quantity of life
- Study Results—life expectancy
  - Reference population: 77.1 years
  - Patients with GD1: 68.2 years
- **For patients with GD1, life expectancy at birth is decreased by about 9 years**



## Neuronopathic GD

- **Type 2**
  - Strabismus
  - Trismus
  - Supranuclear gaze palsy
  - Retroflexion of the neck
  - Limb rigidity
  - Seizures
- **Type 3**
  - Horizontal saccadic abnormalities
  - Retinal infiltrates
  - Strabismus
  - Ataxia





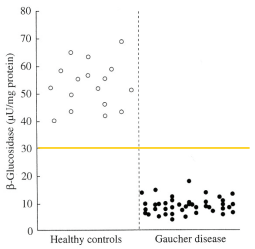
## Diagnosis by a Simple Blood Test

**Acid β-glucosidase assay**

- Can be done on leukocytes (peripheral blood) or cultured fibroblasts (skin biopsy)
- Enzyme activity levels
  - Adults: usually **10% to 30%** of normal
  - Children (severe cases): < 10% of normal
- Residual activity does not predict clinical outcome

**DNA analysis**

- Reliable way to test carriers among relatives at risk
- Genotype does not predict clinical phenotype



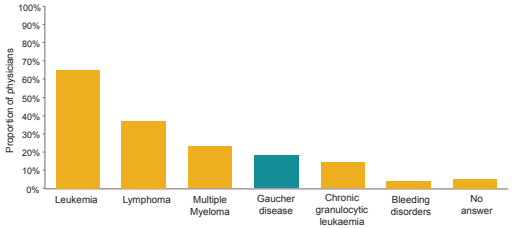

**Leukocyte β-glucosidase activity (μ units (U)/mg protein) of the lymphocyte/monocyte fraction of peripheral blood in 42 patients with GD and 17 healthy controls**



## Diagnosing GD
### Differential Diagnosis

- **Only 1 out of 5 hematologists suspected GD in a patient with 6 of common signs and symptoms of GD.**

**Conditions that physicians suspected when presented with a case of a 42-year old male presenting with anemia, thrombocytopenia, hepatomegaly, splenomegaly, acute bone pain, and chronic bone pain**






## Diagnosing GD: Why is GD missed?
### Differential Diagnosis—Hematologic Diseases

| | GD 1 | CML | ALL | NHL | MM | ITP |
|---|---|---|---|---|---|---|
| **Typical age at diagnosis** | 0-80+ | 2-3 (children) > 65 (adults) | 63 | 10 | 70 | 30-40 |
| **Bone pain** | ✓ | ✓ | ✓ | ✓ | ✓ | |
| **Bruising/ bleeding** | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| **Fatigue** | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| **Hepatomegaly** | ✓ | ✓ | ✓ | ✓ | Less common | Rare |
| **Splenomegaly** | ✓ | ✓ | ✓ | ✓ | Less common | Rare |
| **Gaucher cells on biopsy** | In clusters | Pseudo-Gaucher cells | Pseudo-Gaucher cells | Pseudo-Gaucher cells | Pseudo-Gaucher cells | No |

✓ Yes ☐ Sometimes

## ICGG Gaucher Registry (www.gaucherregistry.com)

- This international observational database tracks natural history and outcomes of patients with Gaucher disease.
  - Initiated in 1991
  - Sponsored by Genzyme Corporation
  - Largest observational study of patients with Gaucher disease in the world
  - Contains data from over 6,000 Gaucher disease patients from more than 60 countries
- All patients with a confirmed diagnosis of GD, regardless of treatment status, are eligible to participate in the Registry
  - The ICGG Gaucher Registry's goals are to:
    - Contribute to the medical understanding of GD
    - Improve the quality of care for GD patients worldwide

Source: ICGG Gaucher Registry



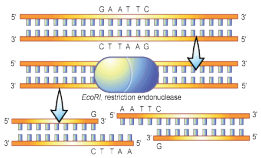
## Biotechnology

Dolly, 1996




## Recombinant DNA Technology

- 1970's – restriction enzymes isolated
- Their function is selective cutting of DNA chain to particular sequences




- Humane insulin (approved 1982) and humane growth factor were the first group of biotechnology products for humane medicine.

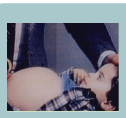


## Genzyme a Sanofi Company

"My mom was just tremendous and an amazing role model for me. I know the doctors told her that I was going to die but her perseverance, dedication and ability to work closely with Genzyme and search around the world to develop a treatment was amazing."

– Brian Berman, Gaucher disease type 1

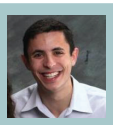
A lifetime commitment



1983

1991

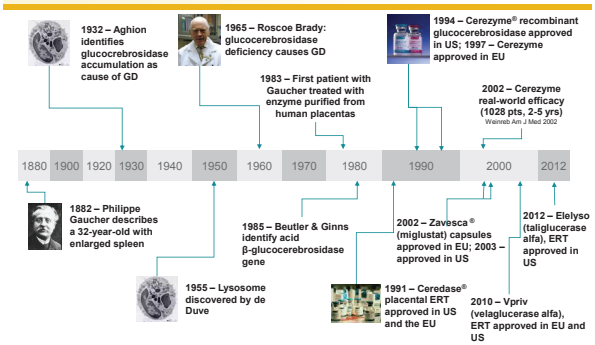
2001



2011



## Gaucher and Therapy Time Line






## Available Disease-Specific Treatments for Gaucher Disease

- **Cerezyme®** (imiglucerase for injection; Genzyme Corporation, a Sanofi company)
  - An analogue of the human enzyme ß-glucocerebrosidase, produced by recombinant DNA technology
  - Indicated for long-term enzyme replacement therapy for pediatric and adult patients with a confirmed diagnosis of Type 1 Gaucher disease that results in one or more of the following conditions:
    a. anemia
    b. thrombocytopenia
    c. bone disease
    d. hepatomegaly or splenomegaly

**Please see full prescribing information. For more information on Cerezyme, visit www.cerezyme.com or call Genzyme Medical Information at 1-800-745-4447.**

Source: Cerezyme SmPC



## Available Disease-Specific Treatments for Gaucher Disease

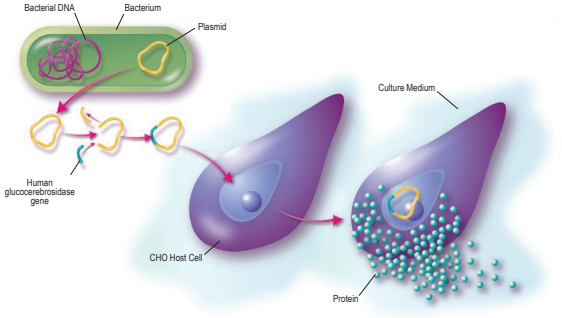
- **VPRIV®** (velaglucerase alfa for injection; Shire Human Genetic Therapies)
  - A hydrolytic lysosomal glucocerebroside-specific enzyme
  - Indicated for long-term enzyme replacement therapy (ERT) for pediatric and adult patients with type 1 Gaucher disease.
- **Elelyso®** (taliglucerase alfa for injection; Pfizer)
  - A hydrolytic lysosomal glucocerebroside-specific enzyme
  - Indicated for long-term enzyme replacement therapy (ERT) for adults with a confirmed diagnosis of Type 1 Gaucher disease
  - Approved in the US and Israel
- **Zavesca®** (miglustat capsules)
  - An N-alkylated imino sugar that inhibits glucosylceramide synthase, an enzyme responsible for the first step in the synthesis of glucosylceramide and other glycosphingolipids, produced by Actelion Pharmaceuticals
  - Indicated for the treatment of adult patients with mild to moderate type 1 Gaucher disease for whom enzyme replacement therapy is not a therapeutic option (e.g., due to constraints such as allergy, hypersensitivity, or poor venous access).

**Please see full prescribing information.**

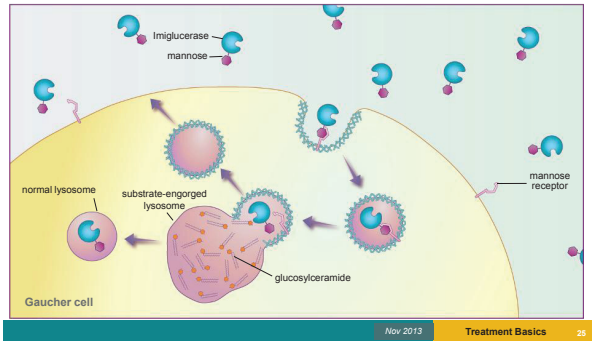
Source: VPRIV, Elelyso, Zavesca SmPCs



## Manufacture of Recombinant Glucocerebrosidase: Imiglucerase



## Imiglucerase: Entry into Lysosomes of Macrophages via Mannose Receptors





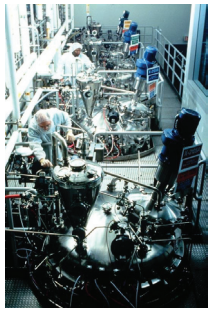

## ERT - Development Costs

- ERT development can take 10+ years and cost hundreds of millions of dollars.
- Investment is high-risk. Only about 10% of orphan drugs eventually get approved.
- Significant costs can continue even after approval.
- ERTs are used by far fewer patients that most other drugs.



## ERT - Manufacturing

- Manufacturing ERTs is a highly complex, resource-intensive, and time-consuming endeavor.
- Genzyme has invested more than $1 billion in manufacturing facilities around the world.
- Genzyme has manufacturing facilities dedicated for each of its ERTs.





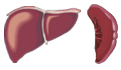
## Imiglucerase Dosage and Administration

- Administered by intravenous infusion over 1-2 hours
- Dosage should be individualized to each patient
  - Initial dosages range from 2.5 U/kg 3 times a week to 60 U/kg once every two weeks
  - 60 U/kg once every two weeks is the dosage for which the most data are available
  - Disease severity may dictate that treatment be initiated at a relatively high dose or relatively frequent administration
  - Dosage adjustments should be made on an individual basis and may increase or decrease, based on achievement of therapeutic goals as assessed by routine comprehensive evaluations of patient's clinical manifestations

Please see full prescribing information. For more information, visit www.cerezyme.com or call Genzyme Medical Information at 1-800-745-4447.



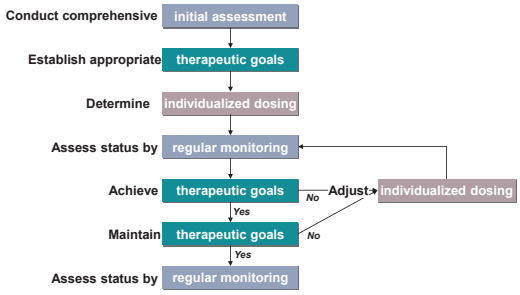
## Key Gaucher Disease Therapeutic Goals After 12-24 Months Cerezyme Treatment



- Skeletal
  - Prevent bone crisis
  - Lessen or eliminate bone pain
- Hematologic
  - Hemoglobin
    - ≥11g/dL (females & children)
    - ≥12 g/dL (males)
  - Platelets
    - >120,000/mm$^3$
  - Reduce fatigue
  - Eliminate blood transfusion dependency
- Visceral
  - Spleen volume ≤ 8 MN
  - Liver volume ≤ 1.5 MN
- QoL: Improve validated QoL scores within 2-3 years

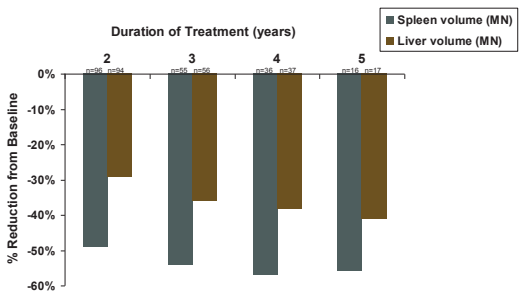


## A Disease Management Algorithm



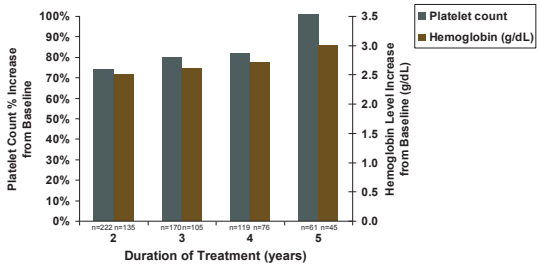



## Imiglucerase Reduces Liver and Spleen Volumes

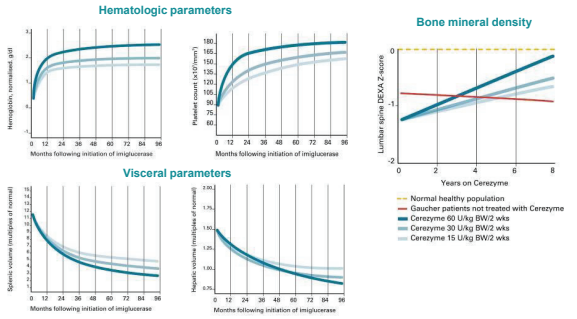





## Imiglucerase Increases Platelet Counts and Hemoglobin Levels



## Imiglucerase Dose-Response Effect

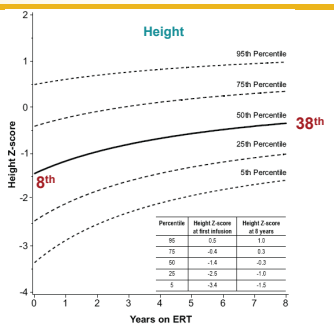



Grabowski GA et al., Genet Med 2009;11:92 and Wenstrup RJ et al., J Bone Miner Res 2007;22:119



## Pediatric Population: Improvement in Pediatric Growth Retardation

Pediatric patients with GDare on average at the 8th percentile for height when starting Cerezyme but at the 38th percentile on average after 8 years of therapy

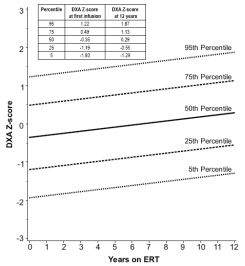



Andersson et al, Pediatrics 2008;122(6):1182-1190S

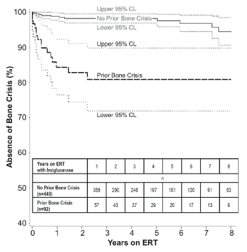


## Pediatric Population: Improvements in Bone Disease

**Improvement in bone mineral density**




**Reduction in bone crisis**




**Children treated with imiglucerase showed improved BMD and reduced rates of bone crisis over 8 years of therapy.**

Andersson et al, Pediatrics 2008;122(6):1182-1190S



## Adverse Events*

| Associated with Route of Administration (IV) | Suggestive of Hypersensitivity | Additional Events |
|---|---|---|
| Discomfort | Anaphylactoid reaction | Nausea |
| Pruritus | Pruritus | Vomiting |
| Burning | Flushing | Abdominal pain |
| Swelling | Urticaria | Diarrhea |
| Sterile abscess at the site of venipuncture | Angioedema | Rash |
| | Chest discomfort | Fatigue |
| | Dyspnea | Headache |
| | Coughing | Fever |
| | Cyanosis | Dizziness |
| | Hypotension | Chills |
| | | Backache |
| | | Tachycardia |

*~13.8% of patients reported adverse events. Each occurred in <1.5 % of the total patient population.

**Please see full prescribing information. For more information, visit www.cerezyme.com or call Genzyme Medical Information at 1-800-745-4447.**



## New Approaches to Treating Gaucher

- New small molecule therapies
  - Enhanced substrate reduction intended to provide better efficacy with fewer side effects: currently in clinical development
  - Pharmacologic chaperones intended to deliver mutant enzyme to lysosomes: clinical development currently suspended
  - Small molecules that could cross the blood-brain barrier and treat neuronopathic disease: research is ongoing
- New delivery systems for enzyme replacement
  - Aimed at addressing neurologic symptoms in neuronopathic GD
  - Research is ongoing
- Gene therapy
  - The "gold standard" approach—could cure the underlying disease
  - Research is ongoing but studies in humans probably still a long way off



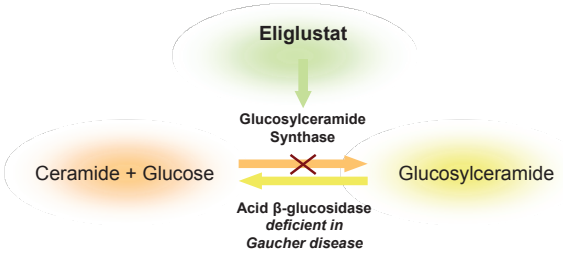
## Treatments in Development for GD

- Eliglustat (Genzyme Corporation, a Sanofi company)
  - Investigational oral therapy in Phase 3 development for the treatment of GD1
  - Potent and specific substrate reduction therapy (SRT)



## Eliglustat Mechanism of Action

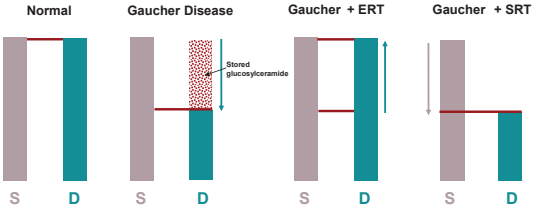
Eliglustat inhibits glucosylceramide synthase, resulting in decreased production of glucosylceramide.






## Enzyme Replacement Therapy (ERT) and Substrate Reduction Therapy (SRT)

**Gaucher Disease: Imbalance between synthesis (S) & degradation (D) of glucosylceramide**



## Bone Mineral Density after 52 weeks
## Mean Lumbar Spine DXA Scores (n=18)

| | Baseline | Week 52 | Change | 95% CI | P-value |
|---|---|---|---|---|---|
| **T-score** (relative to young normal gender-matched control) | | | | | |
| Mean ±SD | -1.77 ±1.057 | -1.39 ±1.029 | **0.38** ±0.521 | 0.12 - 0.64 | **0.0068** |
| Median | -1.95 | -1.25 | 0.35 | | |
| Min, Max | -3.20, 0.60 | -3.00, 0.50 | -0.10, 2.00 | | |
| **Z-score** (relative to age- and gender-matched control) | | | | | |
| Mean ±SD | -1.32 ±1.018 | -1.02 ±1.060 | **0.31** ±0.477 | 0.07 - 0.54 | **0.0146** |
| Median | -1.55 | -0.95 | 0.30 | | |
| Min, Max | -2.80, 0.70 | -2.80, 0.70 | -0.20, 1.70 | | |



## Phase 2 Safety:
## Drug-related and Serious Adverse Events

- Through 1 year of treatment, 7 drug-related AEs were reported in 6 patients
  - More frequently reported at initiation of therapy
  - Headache (1), abdominal pain (2), diarrhea (2), palpitations (1)
  - 1 drug-related adverse event classified as serious: asymptomatic non-sustained ventricular tachycardia (NSVT)
    - Classified as “serious” since patient was admitted overnight for continued telemetry monitoring; patient was discharged the next day after an uneventful stay
    - Assessed as possibly related to study drug by investigator
    - Assessed as unlikely related by 3 cardiologists

Abstract Meeting in Skopje, 16./17.11.2013

**KATRIN KOLLMANN**

Department of Biochemistry, Children's Hospital, University Medical Center Hamburg-Eppendorf, Germany.

## THE LYSOSOMAL STORAGE DISORDER MUCOLIPIDOSIS TYPE II – PATHOMECHANISMS AND THERAPEUTIC STRATEGIES

More than 50 soluble acid hydrolases are involved in the degradation of cellular and extracellular macromolecules and pathogenic organisms in the lysosomes. The constitutive transport of newly synthesized lysosomal proteins from the rough endoplasmic reticulum (ER), via the Golgi apparatus to the endosomal/lysosomal compartment is essential for the biogenesis and functional activity of lysosomes. The majority of soluble lysosomal proteins are modified with mannose 6-phosphate (M6P) residues on N-linked high mannose-type glycans, allowing their recognition by M6P receptors in the Golgi and their subsequent delivery to the endosomal/lysosomal system. The first step in the formation of the M6P-recognition marker on lysosomal hydrolases is catalyzed by the Golgi-resident GlcNAc-1-phosphotransferase, a hexameric complex ($\alpha_2\beta_2\gamma_2$) transferring GlcNAc-1-phosphate from UDP-GlcNAc to specific C6 hydroxyl groups of mannoses. Mutations in the genes encoding the GlcNAc-1-phosphotransferase subunits result in the rare lysosomal storage disorders mucolipidosis II (MLII) and III. Biochemically these diseases are characterized by a hypersecretion and intracellular deficiency of lysosomal hydrolases.

We have generated a mouse model with a common MLII patient mutation which shows growth retardation, skeletal abnormalities, brain atrophy, elevated lysosomal enzymes in serum, lysosomal storage in fibroblasts and brain, and premature death closely mimicking the human MLII disease. We use our mouse model to identify pathomechanisms and to analyze experimental therapies for this rare disease.

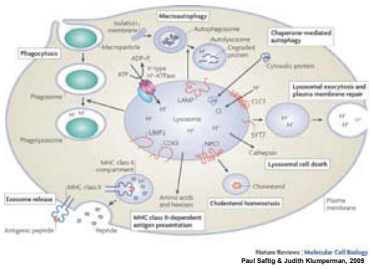
Universitätsklinikum Hamburg-Eppendorf

# The lysosomal storage disorder mucolipidosis type II- Pathomechanisms and therapeutic strategies

**Katrin Kollmann**

Department of Biochemistry,
Children`s Hospital
University Medical Center Hamburg-Eppendorf

## The endosomal/lysosomal compartment



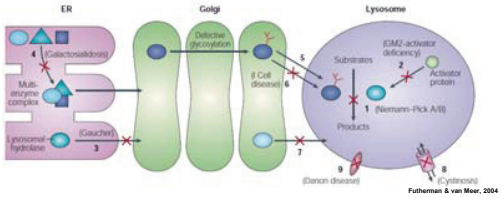
- acidic compartment
- comprising hydrolases
- degradation of macromolecules

Lysosomes are involved in…

- Cholesterol homoestasis
- Modulation of signaling
- Antigen presentation via MHCII
- Pigmentation
- Bone remodelling

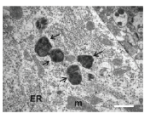
Nature Reviews | Molecular Cell Biology
Paul Saftig & Judith Klumperman, 2009

## Lysosomal storage diseases



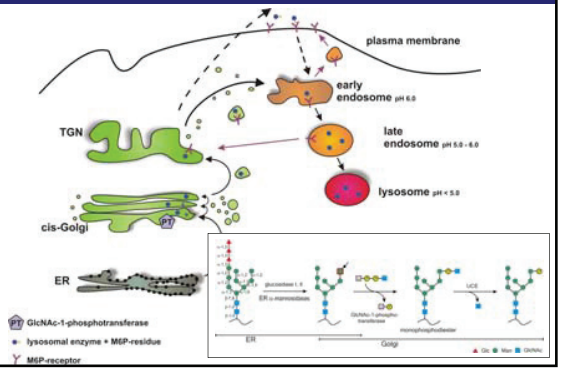

Futherman & van Meer, 2004

- Defects in lysosomal hydrolases, transmembrane proteins, modifying factor lead to the accumulation of undegraded metabolites
- group of approximately 50 rare inherited metabolic disorders
- incidence of single disease ~ 1:100.000, as group ~ 1:7.000

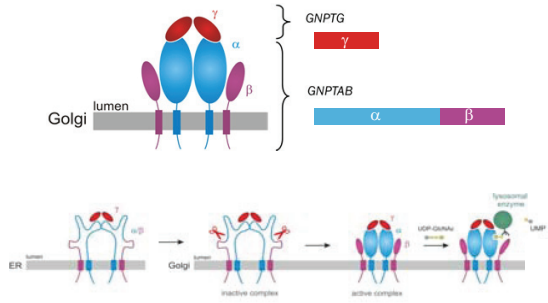



Lysosomal storage in neuron of MLII mice
Kollmann *et al.* 2012

## M6P-dependent transport of acid hydrolases to the lysosomes

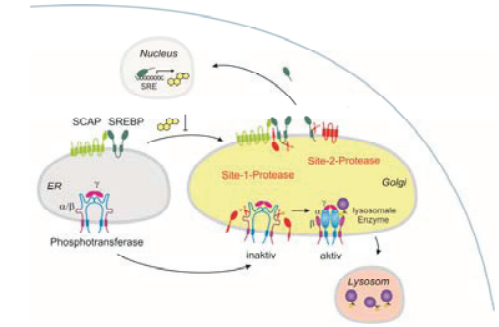



## The GlcNAc-1-phosphotransferase complex



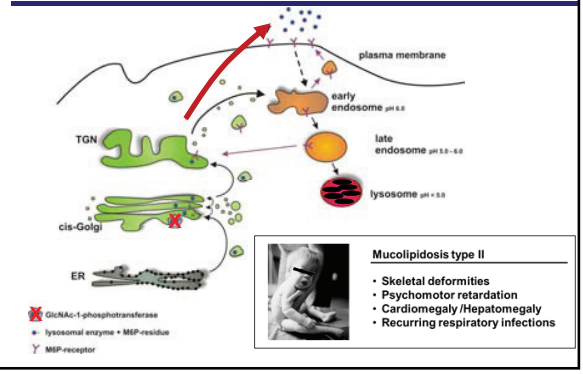

Marschner *et al.* 2011

## The phophotransferase activity is dependent on a protease that regulates cholesterol metabolism




Marschner *et al.* 2011

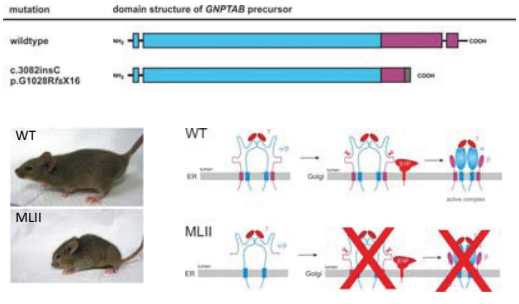
## Deficiency of the GlcNAc-1-phosphotransferase




**Mucolipidosis type II**

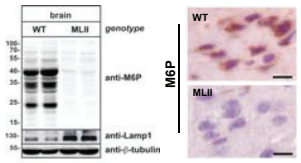
- Skeletal deformities
- Psychomotor retardation
- Cardiomegaly /Hepatomegaly
- Recurring respiratory infections

## The *Gnptab*$^{c.3082insC}$ mouse model for mucolipidosis type II

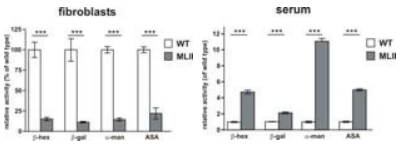


## The loss of phosphotransferase activity leads to a missorting of lysosomal enzymes

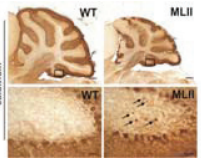
Analysis of lysosomal proteins using an anti-M6P scFv antibody

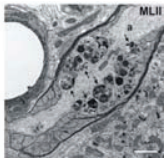



Intracellular deficiency of lysosomal enzymes



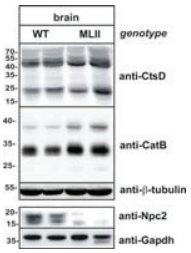

## Neurodegeneration and accumulation of storage material in MLII mice


- Which metabolites are accumulated in the storage material?
- Which hydrolases are absent in neuronal lysosomes inhibiting degradative processes?

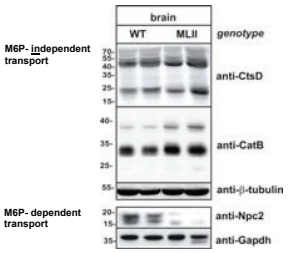
## M6P-dependent and M6P-independent transport to the lysosomes



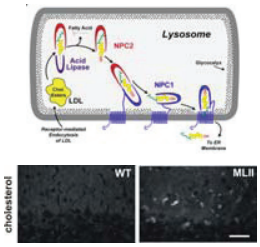

**M6P- independent transport**

**M6P- dependent transport**

## Lysosomal deficiency of Npc2 proteins lead to storage of cholesterol

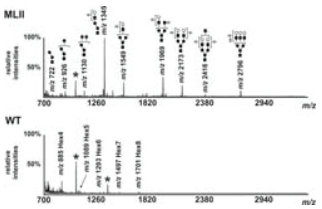


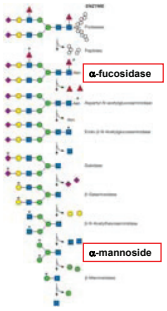

**NPC2 is involved in the transport of cholesterol out of the lysosomes**




## Lysosomal deficiency of α-fucosidase and α-mannosidase

Mass spectrometric identification of free sugar species

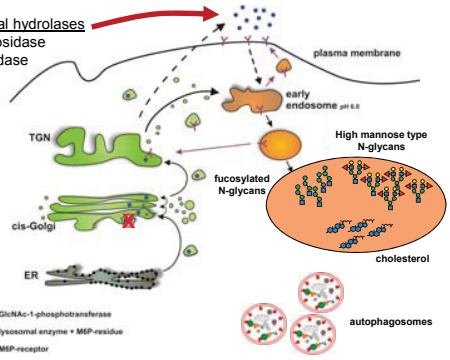



Essentials of Glycobiology

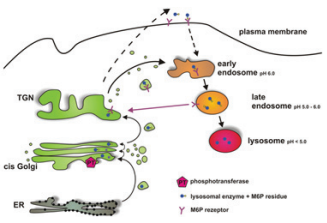
## Summary 1

lysosomal hydrolases
α-mannosidase
α-fucosidase

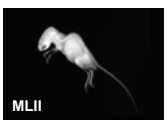


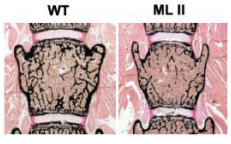

## Therapeutic strategies for lysosomal disorders

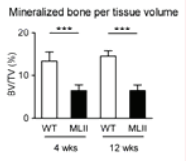
- Enzyme replacement
- Bone marrow transplantation
- Molecular chaperones
- Substrate reduction
- Storage reduction
- Gene therapy
- Symptomatic treatment



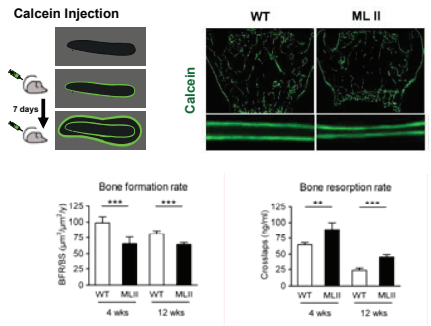

## Skeletal abnormalities in MLII mice

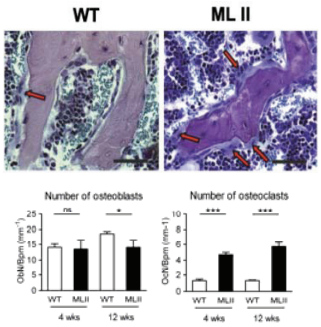



## Analysis of bone formation and bone resorption *in vivo*

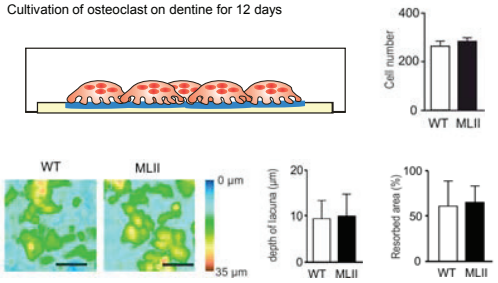



## The number of osteoclasts is highly increase in MLII mice

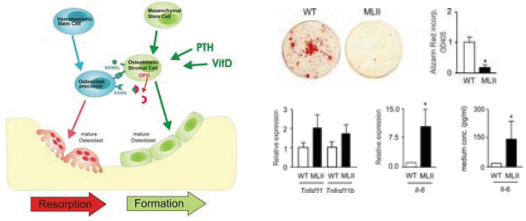



## MLII osteoclasts are not functionally impaired

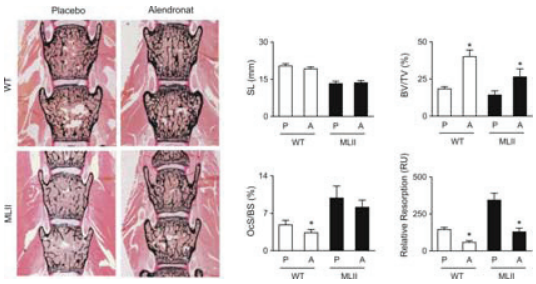
Cultivation of osteoclast on dentine for 12 days



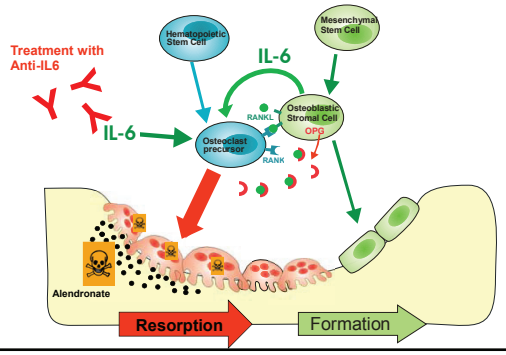

## The osteoclastogenic cytokine IL-6 in highly expressed in MLII bone cells




## Antiresorptive therapy reduces osteopenia in MLII mice




## Summary 2 – Options for symptomatic treatment of the skeletal alterations




## Literature

**Kollmann K**, Pestka JM, Kühn SC, Schöne E, Schweizer M, Karkmann K, Catala-Lehnen P, Failla AV, Marshall RP, Krause M, Santer R, Amling M, Braulke T, Schinke T. Low bone mass due to increased osteoclastogenesis in mucolipidosis II. *EMBO Mol Med.* 2013. doi: 10.1002/emmm.201302979. [Epub ahead of print]

Schweizer M, Markmann S, Braulke T, **Kollmann K.** Ultrastructural analysis of neuronal and non-neuronal lysosomal storage in mucolipidosis type II knock-in mice. *Ultrastruc Pathol.* 2013. 37(5): 366-72

**Kollmann K**, Damme M, Markmann S, Morelle W, Schweizer M, Hermans-Borgmeyer I, Röchert AK, Pohl, S, Lübke T, Michalski JC, Käkelä R, Walkley SU, Braulke T. Lysosomal dysfunction causes neurodegeneration in mucolipidosis II 'knock-in' mice. *Brain.* 2012. 135(9): 2661-75

Marschner K, **Kollmann K**, Schweizer M, Braulke T, Pohl S. A key enzyme in the biogenesis of lysosomes is a protease that regulates cholesterol metabolism. *Science.* 2011. 333:87-90

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**Kollmann K**, Pohl S, Marschner K, Encarnação M, Sakwa I, Tiede S, Poorthius BJ, Lübke T, Müller-Loennies S, Storch S, Braulke T. Mannose phosphorylation in health and disease. *Eur J Cell Biol.* 2010. 89:117-23

Online information

OMIM: http://omim.org/entry/252500
National MPS Society USA: http://www.mpssociety.org
ICLD, UKE, Hamburg: http://www.icld-hamburg.de/

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## SINDROMATIC CAKUT

Congenital anomalies of the kidneys and urinary tract (CAKUT) are important etiological factor in children and adults with chronic kidney disease and end stage renal failure. Anomalies may present isolated or syndromatic. Sometimes only few mild extrarenal abnormalities are evidenced in children with CAKUT (olygosyndromatic CAKUT). CAKUT is present in well known genetic syndromes e.g in a female with short stature and horseshow kidney one should consider Turner syndrome. Even minimal extrarenal abnormalities such as preauricular appendix or polythelia may point to CAKUT (although in the literature there is controversy about this association). In patients with syndromatic features it is mandatory to perform ultrasound scanning of the urinary tract and vice versa in children with well defined CAKUT one should perform detailed physical examination in order to define syndromatic CAKUT. With detection of extrarenal abnormalities one should implement early surgical or conservative interventions. In children with malformations of the auricle, prearicular pits or tags one should suspect BOR-syndrome which may present with nephropathy (renal hypodysplasia, reflux). With early diagnosis of this syndrome one may initiate early hearing rehabilitation and active nephro/urological management. Nowadays the genetic basis of many syndromatic CAKUT is well known (BOR syndrome, renal-coloboma syndrome, renal cyst-diabetes syndrome, Townes-Brocks syndrome, Fraser syndrome, HDR syndrome, tricho-rhino-phalangeal syndrome etc). Modern genetic techniques such as whole exome sequencing or molecular karyotypisation improved diagnostics of syndromatic CAKUT. This enables early and appropriate diagnosis, screening of the family relatives, prenatal diagnosis and early multidisciplinary rehabilitation of the affected individuals.

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*University Pediatric Clinic, CF Center, Skopje, R.Macedonia*

## CYSTIC FIBROSIS: PREVALENCE OF LOW BONE MASS AND VITAMIN D DEFICIENCY IN PEDIATRIC AND ADULT CF PATIENTS IN R.MACEDONIA

Bone disease in cystic fibrosis (CF) has become a topic of widespread interest and impact in the CF community. Recently, some biochemical markers have been proposed to provide information about the dynamics of bone turnover. Only limited information is available for young patients. Imbalance between bone formation and degradation in CF especially in puberty has become an important issue for developing osteopenia. Influence of vitamin D receptor alleles on BMD suggests that these polymorphisms have a greater influence on BMD in childhood. The aim of our study was to assess prevalence of vitamin D deficiency and osteopenia in pediatric and adult CF patients. Methods: The study included 75 clinically stable CF patients (range 5-36 y), who regularly attended CF center at the Pediatric Clinic in Skopje, Macedonia. Serum osteocalcin (OC), β cross laps, 25OHD and PTH were determined by ELISA assays. BMD was measured via dual energy-ray absorptiometry (DEXA) scans with spinal scores recorded. Results: 50 % of the CF patients with PI had serum vitamin D >20 ng (range 10-45ng/ml) with no difference of age. Osteopenia was determined in 35 % of patients. High plasma β cross laps values reflects raised osteoclast activity in 50% of patients with osteopenia. We found one CF patient homozygote for TaqI and Bsml, one for TaqI and one for Fokl. These patients have vitamin D deficiency and osteopenia. Conclusions: Bone remodeling in CF patients is impaired. Further investigations are needed to find underlying pathogenesis of low bone mass and vitamin D deficiency.

## Cystic Fibrosis: Prevalence of low bone mass and vitamin D deficiency in pediatric and adult CF patients in R. Macedonia

Mr.sci.Dr.Jakovska-Maretti, Prof.Dr.sci.S.Fustik
University Pediatric Clinic, Skopje, Macedonia

## Cystic fibrosis (CF)

- Cystic fibrosis is most frequent rare autosomal recessive lethal disease in Caucasian people.
- It is caused by mutation of the gen for cystic fibrosis transmembrans regulator (CFTR).
- Incidence is 1: 2500 newborns
- Mutation in CFTR gene result in defect chloride transport in epithelial cells in pancreas, gut, liver, lung, renal, bone and testicular canals.

## Clinical presentation in CF

- Chronic lung disease with reccurent infections who leads to respiratory insuficiency and eventualy lethal end.
- Malapsorption presented with frequent and oily stools, which are manifestation of pancreatic insufficiency.
- Malnutrition is being an important determinant of growth and body development during childhood and adolescence.

## Actual problem

- Attainment of adulthood is now common in CF and survival continues to increase.
- Major complications emerging with longer survival are failure to maintain body mass, osteoporosis, diabetes mellitus and infertility.
- Incidence of bone disease in CF in the world is 30%.

## Factors who influence over bone mass

- Malnutrition and pancreatic insufficiency
- Deficit of vitamin K
- Deficit of vitamin D
- Polymorphism of VDR gene
- Delayed puberty and hipogonadism
- Physical activity
- Recidivant respiratory infections
- Use of corticosteroids

## Deficit of liposolubile vitamins (A,E,K,D)

- CF patients have low levels of 25OHD because of reduced receiving vitamin D, malapsorption, low levels of vit. D binding protein, reduced sun exposure or rapid catabolism of 25OHD.
- Vitamin K is vital in process of decarboksilaton of osteokalcin (protein of bone formation

## Malresorption

- In CF patients is found reduced absorption of calcium because of deficiency of vit.D and loss of fat free mass (FFM).
- In most of the studies was reported normal concentration of calcium in CF patients.
- Mortenson, Henderson and Salamoni did not find relationship between low BMD and $Ca^{++}$

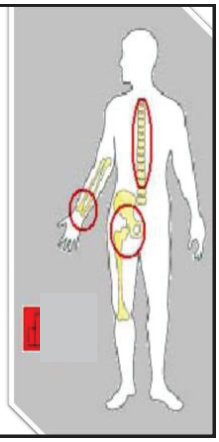
## Bone turnover

- Imbalance between bone formation and degradation in cystic fibrosis (CF) in childhood has become an important issue for developing osteopenia.
- Vitamin D who's activity is determined by VDR gene has influence over bone mass.
- Variants of alleles of VDR gene are ApaI (allele A/a), BsmI (allele B/b), FokI (allele F/f) ,TaqI (allele T/t).

## Fractures

- Risk for fractures in CF patients is higher in late adolescents, especially in female patients.
- Mostly there are fractures on spine (L2-L4).
- Prevalence of radiological vertebral and nonvertebral fractures in the world is 14%.

- vertebral fractures
- fractures of femoral neck
- fractures of distal arm



## AIM OF THE STUDY

- To assess the plasmatic levels of 25OHD in patients with CF who regular visit the CF center at the University Pediatric Clinic in Skopje, Macedonia despite the daily supplementation of 800 IU vitamin D.
- To assess prevalence of vitamin D deficiency and osteopenia in pediatric and adult CF patients.
- To assess bone formation and resorption process with bone markers in prepubertal, pubertal and young adult CF patients.

## METHODS

- The study included 75 clinically stable CF patients (range 5-36 y).
- Serum osteocalcin (OC), β cross laps, 25OHD and PTH were determined by ELISA assays.
- BMD was measured via dual energy-ray absorptiometry (DEXA) scans with spinal scores recorded.
- Variants of alleles of VDR gene were investigated in Institute for imunology and human genetic, Medical Faculty, Skopje

- **Nutritional parameters:**
  - Weight
  - Height
  - W/H expressed as Z score
  - Body mass index (BMI) kg/m$^2$
- **Functional parameters:**
  - FEV1
  - FVC
  - Shwachman-Kulczycki (S-K) *score* is a system for clinical evaluation of CF patients. Maximum score 100 poens.

## Biochemical markers

- Calcium , Phosphorus, Alkaline phosphatasis in serum
- 25OHD was determined with RIA method.
  Referral values for vit.D are 15-60 ng/ml. Values in winter months may be lower for 40-50%.
  In our subjects vitamin D was determined during spring and summer.

## WHO Definition for osteoporosis

Golden standard for definition for osteoporosis:

- Normal : BMD over -1 SD
- Osteopenia: when BMD is between -1SD and -2 SD
- Osteoporosis: when BMD is lower than -2 SD

## Control group

- Control group are 60 healthy voluntears with similar caracteristics.
- They were investigated for calcium, phosphorus, alkaline phosphatase, PTH, osteokalcin, βcrossLaps and 25OHD.

## RESULTS

• 50 % of the CF patients with PI had serum vitamin D >20 ng (range 10-45ng/ml) with no difference of age.

• Osteopenia (Z or T score < -1SD) was determined in 35 % of patients. High plasma β cross laps values reflects raised osteoclast activity in 50% of patients with osteopenia.

• There was one CF patient homozygote for TaqI and BsmI, one for TaqI and one for FokI. These patients have vitamin D deficiency and osteopenia. Further investigations are needed.

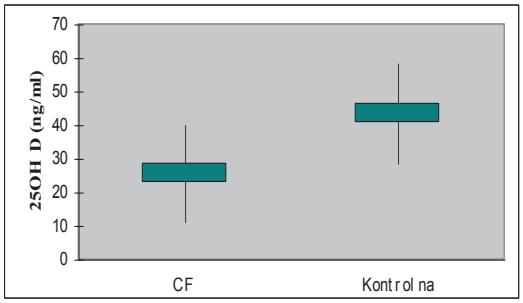
Table 1.: Total number and average age of CF groups of patients

| | 5-11 y. (prepuber. group) | 12-17 y. (pubertal group) | Above 18 y. (adult group) |
|---|---|---|---|
| Total number | 34 | 24 | 19 |
| Average years | 8.32±1.89 | 14.04±1.9 | 23.6±3.5 |

Table 2.: *t*-test for 25OHD in serum between prepubertal, pubertal and adult CF patients

| groups | t-test | p |
|---|---|---|
| Adult/pubertal | 0.55 | 0.58 |
| Adult/prepubertal | -0.76 | 0.44 |
| Pubertal/prepubertal | -1.43 | 0.15 |

Mean value of 25OHD in CF group was 25,56 +/-14,18 ng/ml, and in control group was 43,4 +/-14,9 ng/ml. (p = 0,00034)




## Vitamin D deficiency

- In CF group we found 5 CF patients (16,6%) with 25OHD <10 ng/ml, or 9 (30%) < 15 ng/ml.
- Levels of 25OHD in CF group were significantly lower than in control group (p<0.0001), despite daily supplementation with 800 IU.
- Conclusion is that CF patients have need of higher doses of vitamin D per day and annually monitoring of 25OHD levels.

35% of CF patients are with osteopenia, 2 patients are with osteoporosis

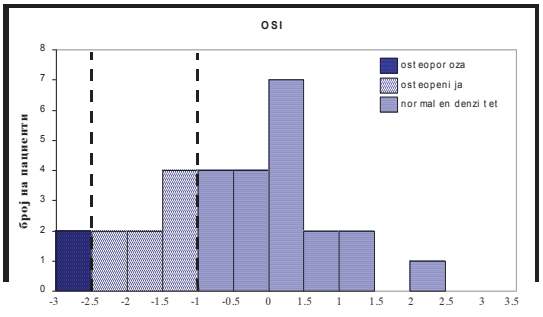



Table 3.: t-test for osteocalcin in serum between prepubertal, pubertal and adult CF patients

| groups | t-test | p |
|---|---|---|
| Adult/pubertal | -5.48 | 0.000003* |
| Adult/prepubertal | -5.01 | 0.000007* |
| Pubertal/prepubertal | 1.99 | 0.05 |

Table 4.: t-test for Þcrosslaps in serum between prepubertal, pubertal and adult CF patients

| groups | t-test | p |
|---|---|---|
| Adult/pubertal | -3.85 | 0.0004* |
| Adult/prepubertal | -3.32 | 0.001* |
| Pubertal/prepubertal | 0.51 | 0.6 |

*statisticaly significant

Table 4.: t-test for Þcrosslaps in serum between prepubertal, pubertal and adult CF patients

| groups | t-test | p |
|---|---|---|
| Adult/pubertal | -3.85 | 0.0004* |
| Adult/prepubertal | -3.32 | 0.001* |
| Pubertal/prepubertal | 0.51 | 0.6 |

*statisticaly significant

Table 5.: t-test for PTH in serum between prepubertal, pubertal and adult CF patients

| groups | t-test | p |
|---|---|---|
| Adult/pubertal | -0.29 | 0.76 |
| Adult/prepubertal | 1.54 | 0.12 |
| Pubertal/prepubertal | 1.44 | 0.15 |

Table 6.: Average values for 25OHD, osteocalcin, ßcrosslaps, PTH, calcium and alkaline phosphatase in serum for prepubertal, pubertal and adult CF patients

| groups | 25OHD | osteocalcin | ßcrosslaps | PTH | Ca | AF |
|---|---|---|---|---|---|---|
| Adult | 21.6 | 28.94 | 0.68 | 49.29 | 2.4 | 240 |
| Pubertal | 20.11 | 94.31 | 1.47 | 53.14 | 2.3 | 230 |
| Prepubertal | 24.04 | 71.2 | 1.37 | 37.68 | 2.4 | 240 |

## CONCLUSION

- There were no difference in bone mineral status or for levels of vitamin D in pediatric and adult CF patients.
- Osteocalcin in pubertal CF patients correlates significantly with the control.
- Levels of markers for bone resorption in serum were elevated in prepubertal and pubertal children with CF.
- This may contribute to impaired bone turnover.

## CONCLUSION

- **There is a possibility of a very early onset of defective bone mineralization in CF independent of severe inflammation and nutritional status.**
- **Bone remodeling in CF patients is impaired.**
- **Further investigations are needed to find underlying pathogenesis of low bone mass and vitamin D deficiency.**

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## BETA THALASSEMIA INTERMEDIA

Beta thalassemias are inherited anemias characterized by decreased or absent synthesis of the beta chains of hemoglobin resulting in variable phenotypes ranging from the clinically silent beta thalassemia minor to completely transfusion dependent beta thalassemia major. The term thalassemia intermedia was suggested almost 60 years ago to describe patients who have clinical manifestations that are too severe to be termed minor, yet too mild to be termed major. Our understanding of the pathophisiological and molecular mechanisms of thalassemia intermedia has significantly increased in the last decade. Despite being considered as a milder form of beta thalassemia, thalassemia intermedia is associated with a variety of serious complications that can increase with age, such as tromboembolic events, pulmonary arterial hypertension and extramedulary hematopoiesis and tumor formation. Therefore, optimal and early intervention is extremely important.

In addition to the phenotypic diversity, thalassemia intermedia is extremely heterogeneous at the genetic level, thus making the molecular diagnosis very challanging. Several patients diagnosed at RCGEB with thalassemia intermedia due to homozigosity of mild beta thalassemia allleles, compound heterozygosity of beta zero and beta silent alleles, coinheritance of alpha thalassemia, and a combination of a beta thalassemia heterozigosity with triplicated alpha globin genes will be presented. A molecular diagnosis of a rare beta thalassemia intermedia patient with mutations in beta and delta globin genes, triplication of alpha globin genes and HPFH will be also presented.

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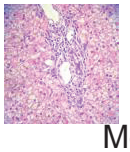
## METABOLIC LIVER DISEASE: A DIAGNOSTIC AND TREATMENT CHALLENGE -EXPERIENCE FROM GASTROENTEROHEPATOLOGY DEPARTMENT

Inherited metabolic disorders (IMD) affecting the liver is a heterogeneous group of rare genetic conditions. The diagnosis is often very difficult to make and the treatment remains a big problem in many cases.

Five patients admitted for liver disorder and diagnosed with IMD at Gastroenterohepatology Department during the past 3 years are presented. The etiologies were: tyrosinemia (HT) type I; glycogen storage disease type IX and III; Gaucher disease and bile acid syntheses defect. The main clinical presentations were: hepatomegaly, jaundice , liver failure. Treatment with nitisinone was provided in tyrosinemia patient, for the first time in Macedonia. The patient with Gaucher disease started treatment one year after diagnosis.. Molecular analysis was performed in 4 patient. Novel splice site mutation was detected in our patient with glycogenosis type IX-b (*IVS18+1G>C)*.

Conclusion: The diagnosis of metabolic disorders is a challenge. High index of suspicion, early diagnosing and prompt management is necessary to avoid unfavorable outcome.



## Metabolic liver diseases - diagnostic and treatment challenge

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University Children,s Hospital
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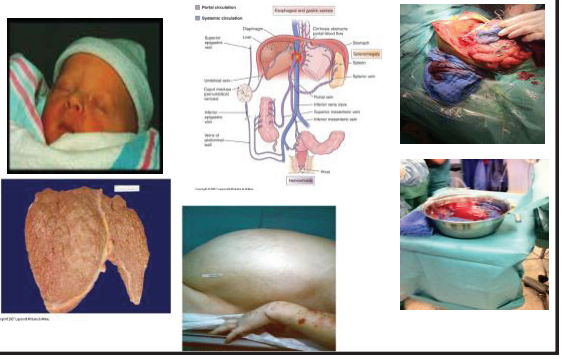
Skopje

## What we want to avoid?



## Clinical presentation of MLD

- **Liver failure**
- Encephalopathy or Reye-like ilness
- Chronic cholestasis
- Isolated hepatomegaly or hepatosplenomegaly

## Liver failure
## Investigations

- Erythrocyte galactose-1-phosphate uridyltransferase
- Plasma and urine amino acids
- Urine organic acids
- Urine succinylacetone
- Plasma alfa-fetoprotein
- Plasma lactate
- Plasma /blood spot acylcarnitines
- Plasma ferritin, TIBC
- Serum alfa-antitrypsin and phenotype

## Case 1. Liver failure

- Female newborn 2.5 month old
- **Hospitalisation**: *Referred because of malnutrition and anemia*
- **History:**
  - Normal gestation BW 3100g
  - Main complaints:failure to thrive, diarrhoea, edema

**Clinical findings**

- Hepatosplenomegaly
- Edema
- Ascites
- Body weight 4800g

## Dg : Tyrosinemia type I

- Alfa feto protein: >10.000IU/ml
- Hipoproteinemia (32g/l), hipoalbuminemija (18g/l)
- Protrombine time 46 sec
- Aminoacidemia : elevated values for tyrosine of 396.
- Positive succynilaccetone in urine
- **Imaging evaluation:**
  - *Ultrasonography* **:hepatosplenomegaly with hyperecchogenic structure. Ascites. Hypoecchogenic structure of medulla of kidneys**

### Treatment

- **For the first time in Macedonia Nitisinone 1mg/kg/bw**
- **Low tyrosine diet**

## Clinical course and outcome at age of 3 years:

- Normal psychomotor development
- Normal coagulation profile
- Normal total proteins/albumin, alfa-feto protein, functional hepatic tests

**-Tyrosine 245 µmol/l**
**-Succynilaccetone: negative**
**- CT of abdomen with normal findings**
**-Molecular analysis: Department of Pediatrics. First Faculty of Medicine, Charles University in Prague : result expecting**

## Conclusion

Our first experience with nitisinone treatment showed promising results improving the prognosis for patients with HT1

- Avoiding of severe liver disease and transplantation

## Clinical presentation of MLD

- Liver failure
- Encephalopathy or Reye-like ilness
- **Chronic cholestasis**
- Isolated hepatomegaly or hepatosplenomegaly

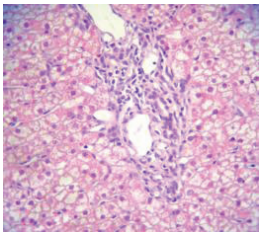
## Chronic cholestasis Investigations

- As for liver failure, plus:
- Plasma very long-chain fatty acids
- Plasmatransferin isoforms
- Vacuolated lymphocytes in peripheral blood
- Storage cells in liver/bone-marrow biopsy
- Consider specific enzyme assay in leukocytes /fibroblasts
- Urine and plasma bile acids

## Case 2 Cholestasis

- male infant, 4 months of age
- mild jaundice since birth
- at 3 months more yellow
- initial labs done in Kosovo
- jaundiced skin and sclerae
- liver **4,5 cm** bellow the costal margin spleen **2 cm** below left costal margin
- Investigations
  - Total bilirubine – 267 µmol/l (**conjugated- 222 µ mol/l )**
  - AST - 850 IU/l , ALT - 857 IU/l, γGT- 155 IU/l

- **Liver biopsy:**
  - giant-cell hepatitis



## Metabolic investigations

(metabolic laboratory in Heidelberg)

- aminoacids in plasma, organic acids in urine no abnormalities
- **total bile acids in serum:**
  - **Clearly elevated concentration** : 249.9 µmol/l (normal 0 – 22)

  **Conclusion:**
  - Highly elevated concentation of total bile acid in serum
- together with the elevated transaminases and normal gamma-GT and giant cell hepatitis diagnosis of **bile acid defect** was made
- **Dg: 5-beta reductase deficiency**

## Treatment

- Chenodeoxycholic acid 8 mg/kg/day
- + Cholic acid 8 mg/kg/day
- **Molecular analysis**
- *(AKR1D1 gene mutation)*
- Patient migrated in Italy

## Clinical presentation of MLD

- Liver failure
- Encephalopathy or Reye-like ilness
- Chronic cholestasis
- **Isolated hepatomegaly or hepatosplenomegaly**

## Hepatomegaly or hepatosplenomegaly Investigations

- Plasma glucose, lactate,urate, lipids
- Urine oligosaccharides
- Urine glycosaminoglycans
- Liver histology
- Consider specific enzyme analysis on liver/leukocites
- Vacuolated lymphocytes
- Storage cells in liver/bone marrow
- Plasma chitotriosidase, transferin isoforms

## Case 3 Hepatomegaly

- A 2.5-year-old boy
- Distended abdomen
- Hepatomegaly
- No symptoms of hypoglycemia
- No muscle involvement.
- Body height at the 8th percentile
- Weight at the 88th percentile.
- Liver enlarged 10 cm
- Spleen not palpable

## Liver function tests

- Elevated aminotransferases
  - AST 355 U/l
  - ALT 156 U/l
- Normal values
  - total bilirubin 9µmol/l
  - γGT 49 U/l

**Ultrasound of the abdomen-**
Marked hepatomegaly

- Daily blood sugar profile ranged from 65 mg/dl to 178 mg/dl, and no hypoglycemia was noticed.
- Serum triglycerides elevated (3,76 mmol/l)
- Cholesterol normal (4,6 mmol/l).
- Lactic acid and uric acid normal
- Percutaneous liver biopsy
  - Enlarged hepatocytes, with swollen, pale cytoplasm giving the impression of so called "plant like hepatocyte morphology".

## Biotinidase elevated

| **Biotinidase** | | **Normal values** | |
|---|---|---|---|
| Biotinidase in serum | **9,75 +** | 2,51-8,11 | nmol/l/min/ml |
| Percent of the normal (BIO) | **182 +** | 49-151 | % |
| Biotinidase_2 in serum | **11.05 +** | 2,54-7,81 | nmol/min/ml |
| Percent of the normal (BIO2) | **211 +** | 50-149 | % |
| Ratio Biotinidase 2/Biotinidase 1 in serum | **1,13** | 0,73-1,25 | |

| **Enzyme** | | **Reference range:** |
|---|---|---|
| Amylo 1-6 Glycosidase, substrate: limit dextrine U/g protein (GSD type III) | 3,5 | 1-6 |
| Amylo 1-6 Glycosidase, substrate: glycogen, U/g protein (GSD type III) | 1,2 | 0,3-3 |
| Liver phosphorylase, U/g protein (GSD type VI) | 18,2 | 5-50 |
| **Phosphorylase-b kinase in erythrocytes, U/g hemoglobin (GSD type IX)** | **< 0,5 !!** | 1-10 |
| **Phosphorylase-b kinase in leucocytes, U/g protein (GSD type IX)** | **< 2,9 !!** | 40-150 |
| Glycose-6-phosphat dehydrogenase in erythrocytes, U/g hemoglobin (control) | 7,2 | 2-15 |

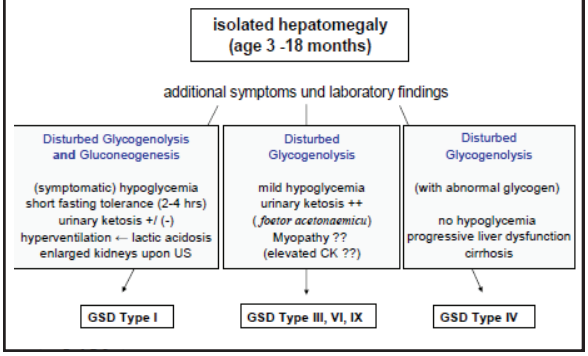
## Two mutations in the *PHKB gene* identified

- Compound heterozygous
- *IVS18+1G>C / c.1969C>T*
  - The first one splice site mutation at the donor splice site of intron 18 (novel mutation)
  - and the other one was in exon 20.
    - The second mutation *c.1969C>T* results with [p.Q657X] a nonsense mutation, already described in GSD type IX patients
    - Molecular analysis: parents
      - Father: Heterozygous for *IVS18+1G>C*
      - Mother:Heterozygous for *c.1969C>T*

## Follow -up

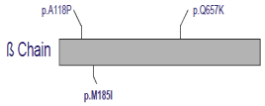
- With proper treatment:
  - Hepatomegaly decreased
  - Hepatic function improved
  - Growth improved

## Diagnostic Approach to Liver GSDs



## Genetics for GSD IX-b

**Type IX-b course could be predicted with mutation analysis**




| Exon | Mutaton | Predicted Effect |
|---|---|---|
| 5 | c.306-2A>G | Deletion of exon 5 |
| 8 | 7574 bp deletion | Deletion of exon 8 |
| 14 | c.1257T>A | p.Y419X |
| 14 | c.1275dupA | p.N422KfsX2 |
| 14 | c.1285C>T | p.R429X |
| 20 | c.1827G>A | p.W609X |
| 21 | c.1969C>T | p.Q657X |
| 27 | c.2337-2A>C | Deletion of exon 27 |
| 31 | c.2926G>T[4] | p.E976X |
| 31 | c.2896-1G>T | c.2896_2911del16 |

## Case 4 hepatomegaly

- Male, 3 years old
- Hepatomegaly
- Short stature <3 percentile for height
- No skeletal myopathy
- Hypoglycemia
- Elevated transaminases
- Hyperlipidemia
- No Cardiomyopathy
- No renal involvement

## Diagnosis

- Fasting hypoglycemia, ketosis/ketonuria
- Hyperlipidemia (cholesterol>6mmol/l)
- Uric acid normal
- Lactate>2.5-5mmol
- Elevated aminotransferases
- CPK elevated
- Positive glycose challenge test: rise of glycemia and serum lactate
- Liver histology:
  - fibrosis
- Confirmed Dg:
  - deficient enzyme (Le/liver tissue)
  - DNA analysis in Hamburg: result expecting

## Enzyme activity result

Patient: **JOVANCHOV, FYLYP** Withdrawal: **15.10.2013**
Born: **15.10.2013** Entr. date: **17.10.2013 12:11**
Gender: **M** Exit date: **25.10.2013 17:10**
Clinical remarks: **final report**

| Test | Result | Unit | Ant. result | Reference range |
|---|---|---|---|---|
| *Glycogen Storage Disease (GSD) profile:* | | | | |
| *Glycogen in erythrocyte:* | *++ 38* | *mg/dl* | | *RR: 0 - 10* |
| *Branching enzyme in erythrocyte:* | *17.0* | *µmol/min/g Hb* | | *RR: 8 - 25* |
| *Phosphyrylase-Kinase in erythrocytes:* | *195* | *µmol/min/g Hb* | | *RR: 100 - 300* |
| *Phosphorylase a in leucocytes:* | *5* | *nmol/min/mg protein* | | *RR: 5 - 20* |
| *Phosphorylase a+b in leucocytes:* | *11* | *nmol/min/mg protein* | | *RR: 10 - 50* |
| *Amyloglukosidase in erythrocytes:* | *! 0* | *nmol/min/g Hb* | | *RR: 0.6 - 3.5* |
| *Biotinidase in plasma:* | *+ 9.5* | *nmol/min/ml* | | *RR: 3 - 8* |

## Glycogenosis type III

- Deficiency:
  - debrancher enzyme amylo-1,6-glukosidase
- Accumulation of limit dextrin
- GSD IIIa (80%)
  - Liver, muscle, fibroblasts, cardiac muscle,Er
- GSDIIIb
  - liver
- Prevention and treatment of hypoglycemia
- Result
  - Catch-up growth
  - Decreased liver size
  - Improved liver function
  - But also, Progressive liver disfunction, LF
- Hepatic adenoma (25%)
- Liver cirrhosis and HCC
- LT for
  - Cirrhosis
  - End stage liver disease
  - HCC

## Case 5. Hepatosplenomegaly

- Gemellus, female 11 years
- Hepatomegaly 4 cm
- Splenomegaly 10 cm
- Abdominal pain
- Bone pains
- Investigations:
  - Anemia, Leucopenia, Trombocitopenia
  - Bone marrow aspiration
    - Gaucher cells present
- Enzyme analysis

Ime i prezime: KURTIŠI AZELINA
Datum rodenja: 7.6.2001.
Lab. broj: 1737
Uzorak od: 26.09.2012.

Klinika/odjel:
UNIVERSITY CHILDREN S HOSPITAL SKOPJE
DR.KOSTOVSKI

**N A L A Z**

☐ LIZOSOMSKI ENZIMI

| Enzim | Bolesnik | Kontrola | |
|---|---|---|---|
| α-GALAKTOZIDAZA | | | *Fabryeva bolest* |
| β-GALAKTOZIDAZA | | | *GM1-gangliozidoza, MPS IVB* |
| β-GLUKOZIDAZA | 0,17 | 8,3 | ***Gaucherova bolest*** |
| α-GLUKOZIDAZA | | | *Pompeova bolest* |
| SFINGOMIJELINAZA | | | *Niemann-Pick A i B* |
| β-HEKSOZAMINIDAZA A i B | | | *GM2-gangliozidoza Sandhofova bolest* |
| β-HEKSOZAMINIDAZA A | | | *GM2-gangliozidoza Tay-Sachsova bolest* |
| GALAKTOCEREBROZIDAZA | | | *Krabbeova bolest* |
| ARILSULFATAZA A | | | *Metakromatska leukodistrofija* |
| α-L-IDURONIDAZA | | | *MPS I* |
| HEPARIN SULFAMIDAZA | | | *MPS IIIA* |
| α-N-ACETILGLUKOZAMINIDAZA | | | *MPS IIIB* |
| N-ACETILGALAKTOZAMIN 6-SULFAT SULFATAZA | | | *MPS IVA* |
| ARILSULFATAZA B | | | *MPS VI* |
| HITOTRIOZIDAZA u serumu | 15 120 | 54 | |

Miljenje : Izrazito snižena aktivnost beta-glukozidaze u homogenatu leukocita uz značajno povišenu aktivnost serumske bitotrioziidaze.

Takav nalaz upućuje na dijagnozu GAUCHEROVE BOLESTI.

## M Gaucher

- The commonest lysosomal storage disease
- Classification:
- Type 1 (nonneuronopathic) 94%
- Type 2 (acute neuronopathic) 5%
- Type 3 (subacute neuronopathic) 1%
- Treatment ERT with cerezyme
  - Started from august 2013 (1 year after Dg)

## Conclusion

- Establishing diagnosis is mostly difficult
- Special examinations unavailable in our country
- Always not available treatment
- Treatment is cheap in some (GSD, bile synthesis defects) or
- Expensive (Gaucher, Tyrosinemia I)
- Prognosis different
- Sometimes liver transplantation

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*Pediatric Clinic*
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## GENETIC BACKGROUND OF PRECOCIOUS PUBERTY

**Apstract**

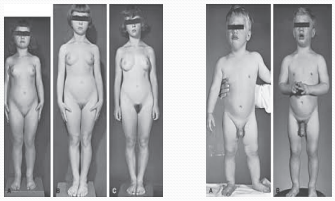
- Precocious puberty is defined as appearance of secondary sexual characteristics before 8 years in girls, and before 9 years in boys
- Today, puberty begins earlier than a few decades ago, and seems to be influenced by enviromental, genetic and racial/ethnic backgrounds
- Central precocious puberty (CPP) may be idiopathic (90%) and organic (10%)
- The term "idiopathic" for CPP was given due to unknown factors influencing the premature activation of the hypothalamic-pituitary-gonadal (HPG) axis
- Genetic factors play a fundamental role in the timing of pubertal onset, as illustrated by the similar age at menarche among members of an ethnic group and in mother-daughter, monozygotic twins and sibling pairs
- It was discovered lately that multiple genes may be involved in the pubertal onset
- KISS1 and KISS1R
- GNRH1
- GNRHR
- LIN28B
- TAC3
- TACR3
- New techniques of DNA sequencing, as whole exomig or genomic sequencing will be helpful in uncovering these still unknown genes

# Genetic background of precocious puberty

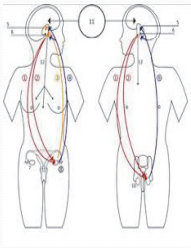
Marina Krstevska-Konstantinova, MD, PhD
Pediatric Clinic
Skopje, Macedonia

## Introduction

- Precocious puberty is defined as appearance of secondary sexual characteristics before 8 years in girls, and before 9 years in boys



- Today, puberty begins earlier than a few decades ago, and seems to be influenced by enviromental, genetic and racial/ethnic backgrounds.



- Central precocious puberty (CPP) may be idiopathic (90%) and organic (10%)
- The term "idiopathic" for CPP was given due to unknown factors influencing the premature activation of the hypothalamic-pituitary-gonadal (HPG) axis

## Genetics

- Genetic factors play a fundamental role in the timing of pubertal onset, as illustrated by the similar age at menarche among members of an ethnic group and in mother-daughter, monozygotic twins and sibling pairs

- It was discovered lately that multiple genes may be involved in the pubertal onset

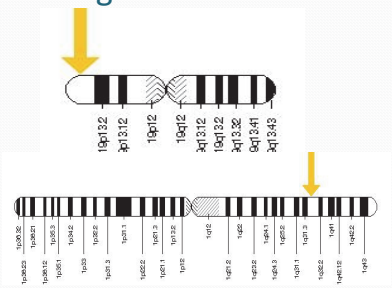
## Candidate genes for CPP in humans

- KISS1 and KISS1R
- GNRH1
- GNRHR
- LIN28B
- TAC3
- TACR3

## Candidate genes for CPP in humans

- **Where is the *KISS1R* gene located?**
- Cytogenetic Location: 19p13.3
- Molecular Location on chromosome 19: base pairs 917,341 to 921,014
- The KISS1R gene is located on the short (p) arm of chromosome 19 at position 13.3.
- More precisely, the *KISS1R* gene is located from base pair 917,341 to base pair 921,014 on chromosome 19.

## Candidate genes for CPP in humans



- Two-gain-of-function mutations in KISS1 and KISS1R have been identified recently as genetic causes of CPP

- In 2003, two groups of scientists individually discovered the presence of deletions and inactivating mutations of KISS1R in patients with idiopathic hypogonadotropic hypogonadism

- Teles et al. identified a heterozygous activating mutation in the KISS1R (p.Arg 386 Pro) in a girl with ICPP

- Silveira et al. (2010) studied 83 children (77 girls) with ICPP and reported a heterozygous KISS1 activating missense mutation (p. Pro 74 ser) in a boy who developed CPP at 1 year of age

- Another KISS1 rare variant (p. His 90 Asp) was identified in three unrelated girls with sporadic CPP

- Ko et al. (2010) studied 101 Korean girls with ICPP. Only known polymorphisms or synonymous changes were detected and none of the mutations in KISS1

- Luan and Zhon (2007) also studied the KISS1 gene in 272 Chinese Han girls with ICPP and did not find mutations

- Tommiska et al. (2011) studied 30 girls with ICPP for mutations in KISS1, KISS1R and LIN28B and no rare variants were identified.

- We did not find in our study, any rare variants in KISS1 and KISS1R in 28 girls with ICPP, corroborating the concept that mutations in the kisspeptin system are a very rare cause of ICPP

## Conclusion

- New techniques of DNA sequencing, as whole exomig or genomic sequencing will be helpful in uncovering these still unknown genes

**SNEZANA JANCEVSKA1,**
*Mile Kitanovski2,*
*Ilija Kirovski3*

PHO University Clinics of Gynecology and Obstetrics, Skopje, R of Macedonia1, 2 University Children's Hospital, Skopje, R of Macedonia3

## EMANUEL SYNDROME – CLINICAL AND MOLECULAR ANALYSIS

Multiple congenital anomalies and craniofacial dysmorphism are characterizing the Emanuel syndrome. An important mental and developmental retardation are major features.

We present a 2 years old child, with normal prenatal history, presented cyanotic at delivery with ear anomalies, preauricular tag, high-arched palate, micrognathia. There was not microcephaly, nor heart defects. Psychological testing confirmed the significant mental and developmental delay. Ultrasound of the kidneys and heart were uneventful.

Karyotype from peripheral blood (G-banding) indicated Emanuel syndrome (a 47,XY,der(22)t(11;22)(q23;q11.2). MLPA analysis of the 11th and 22nd chromosome showed two duplications on the 11th chromosome (4 analysed) and 4 duplications on the 22nd chromosome (11 analysed). Those were the data: 11q25 133292680-133292754 MLPA (P070-B2 Human Telomere-5) duplication, 4 11q25 133595730-133595797 MLPA (P036-E1 HumanTelomere-3) duplication, 22q11.1 15959672-15959739 MLPA (P070-B2 Human Telomere-5) duplication, 6 22q11.21 16606684-16606759 MLPA (P036-E1 HumanTelomere-3) duplication, 7 22q11.21 17891318-17891378 MLPA (P245 Microdeletion-1) duplication, 8 22q11.21 18091521-18091580 MLPA (P245 Microdeletion-1) duplication.

This syndrome is caused by malsegregation of the t(11;22)(q23;q11.2) translocation and is a non-Robertsonian translocations. Unfortunately we were not able to test the parents. Clinical follow up is underway and we would prefer further molecular testing to pinpoint more accurately the molecular defect.

# Emanuel syndrome – clinical and molecular analysis

Snezana Jancevska[1],
Mile Kitanovski[2], Ilija Kirovski[3]

PHO University Clinics of Gynecology and Obstetrics, Skopje, R of Macedonia[1,2]
University Childrens Hospital, Skopje, Macedonia[3]

Emanuel syndrome is a chromosomal disorder that disrupts normal development and affects many parts of the body.

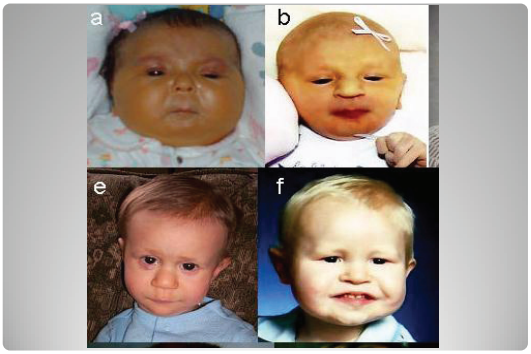
ES is named after Dr Beverly Emanuel, a cytogeneticist in Philadelphia,USA.

Most of the clinical information about this syndrome was published prior to the mid-1980s*

Previously it has been referred to as derivative 22 syndrome, derivative 11;22 syndrome or partial trisomy 11;22.

The exact incidence is unknown. This is a rare syndrome with reported cases of around 100.

* Fraccaro et al., 1980; Zackai and Emanuel, 1980; Iselius et al., 1983; Emanuel et al., 1976; Lin et al., 1986.



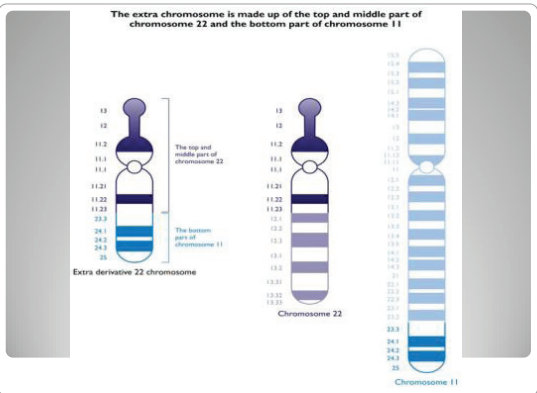

Accepted from: . Carter M.T, Pierre S.A.S, E. Zackai E.H, Emanuel B.S, and Boycott K.S. "Phenotypic delineation of Emanuel syndrome (supernumerary derivative 22 syndrome): clinical features of 63 individuals," *American Journal of Medical Genetics A*, vol. 149,no. 8, pp. 1713–1721, 2009.

ES is caused by the presence of extra genetic material from chromosome 11 and chromosome 22 in each cell. Patients with ES have an extra chromosome consisting of a piece of chromosome 11 attached to a piece of chromosome 22.

The extra chromosome is known as a derivative 22 or der(22) chromosome.

These individuals with unbalanced translocation have three copies of some genes in each cell instead of the usual two copies.
The excess genetic material provocates bad development, intellectual disability and birth defects.

**Researchers are working to determine which genes are included on the der(22) chromosome and what role these genes play in development.**

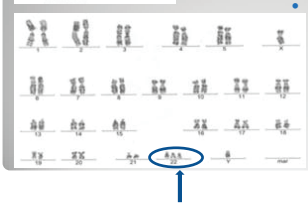



## From case history

- We present a 2 years old child,
- A young mother aged less than 25 years was reported with a male neonate.
- The marriage of the infant's parents was not consanguineous.
- First child from first mature pregnancy, with normal prenatal history,
- Vaginal delivery, with out of complications,
- Full term newborn baby, eutrophic, hypotonic and cyanotic,
- In the begeening of postnatal life, newborn infant has a non typical distinctive phenotype, consisting of characteristic facial dysmorphism.

| | System involved | Clinical features of Emanuel syndrome | Case report |
|---|---|---|---|
| 1 | **Growth and development** | Pre and postnatal growth retardation, delayed speech, and language development | **postnatal growth retardation, delayed speech and language development** |
| 2 | **Craniofacial** | Microbrachycephaly, prominent forehead, epicanthal folds, downslanting palpebral fissures, abnormal auricles, preauricular ear pits and tags 76%, deafness. | **preauricular ear tags, abnormal auricles** |
| 3 | **CNS** | Microcephaly present most commonly, seizures, failure to thrive, and delayed psychomotor development | **failure to thrive, and delayed psychomotor development** |
| 4 | **Cardiac** | 60% congenital heart defects (ASD, VSD, Tetralogy of Fallot, and PDA) | / |
| 5 | **Genitointestinal** | Diaphragmatic hernia, anal atresia, inguinal hernias, biliary atresia, small penis 64%, cryptorchidism 46% | **cryptorchidism** |
| 6 | **Musculoskeletal** | Centrally based hypotonia, congenital hip dislocation, arachnodactyly, club foot and joint, syndactyly of the toes, hyperextensibility of joints | **Centrally based hypotonia** |
| 7 | **Oral findings** | Cleft palate 50%, micrognathia 60%, angular mouth pits, bifid uvula, and facial asymmetry | **Micrognathia facial asymmetry** |
| 8 | **Immunological** | Congenital immunological deficiency | |
| 9 | **Renal** | Renal defects 36% | / |

* Choudhary M.G, Babaji P, Sharma N, Dhamankar D, Naregal G, and Reddy V.S. *Case Report* Derivative 11;22 (Emanuel) Syndrome: A Case Report and A Review. Hindawi Publishing Corporation Case Reports in Pediatrics Volume 2013, Article ID 237935, 4 pages.



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Those were the data:

11q25 133292680-133292754

- MLPA (P070-B2 Human Telomere-5) duplication, 4 11q25 133595730-133595797
- MLPA (P036-E1 HumanTelomere-3) duplication, 22q11.1 15959672-15959739
- MLPA (P070-B2 Human Telomere-5) duplication, 6 22q11.21 16606684-16606759 MLPA (P036-E1 HumanTelomere-3) duplication, 7 22q11.21 17891318-17891378
- MLPA (P245 Microdeletion-1) duplication, 8 22q11.21 18091521-18091580
- MLPA (P245 Microdeletion-1) duplication.

- This syndrome is caused by malsegregation of the t(11;22)(q23;q11.2) translocation and is a non-Robertsonian translocations.
- Unfortunately we were not able to test the parents.
- Clinical follow up is underway and we would prefer further molecular testing to pinpoint more accurately the molecular defect.

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**On line informations:**

OMIM: http://omim.org/entry/609029

GHR: http://ghr.nlm.nih.gov/condition/emanuel-syndrome



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## NOTES