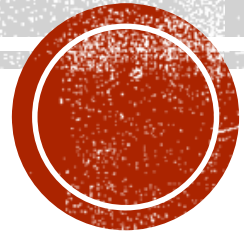
TESTAREA GENETICA IN CENTRUL REGIONAL DE GENETICA MEDICALA CLUJ

Miclea D., Alkhzouz C., Popp R.A., Zimmermann A., Lazar C., Lazea C., Bucerzan S., Farcas M., Grigorescu-Sido P.



OBIECTIV

- Testarea genetica efectuata la Laboratorul de Genetica al Spitalului Clinic de Urgență pentru Copii, Centrul Regional de Genetica Medicala Cluj

INTRODUCERE

- Testarea genetica – diagnostic pozitiv intr-o patologie genetica
- Genetica medicala in urmatorii 15 ani – medicina genomica (medicina de precizie)
  - Diagnostic si evolutie: boli rare, boli comune
  - Tratament: Farmacogenetica

Ghiduri de testare genetica, in Europa - utilizate de ≈ 3-5 ani
- Patologia endocrina: DSD/hipostatura – panel de gene NGS
- Boli genetice de metabolism: panel de gene NGS
- DD/ID, TSA, epilepsie – array CGH/SNP, panel, exom NGS

- Aceasta se poate efectua pentru tot mai multe patologii şi în ţara noastră

TESTAREA GENETICA
CENTRUL REGIONAL DE GENETICA CLUJ

1) patologia endocrină (Miclea D)
- ADS/hiperplazia congenitala de corticosuprarenala şi anomalii ale pubertăţii
- hipostatura şi anumite displazii scheletale
- obezitatea

2) fibroza chistica şi anumite boli genetice de metabolism (Farcas M)
- boala Gaucher, deficitul de alfa1antitripsină

3) patologia senzoriala (surditatea), psihiatrica şi neurologică (Farcas M, Miclea D)
- RD/DI, TSA, epilepsia - forme izolate sau sindromice

METODA

- aparatura
  - Laboratorului de Genetica Medicala al Spitalului Clinic de Urgenta pentru Copii, Centrul Regional Cluj
  - Laboratorului de Genetica Medicala, UMF Cluj
- Parteneriate cu:
  - Centrul Imogen, Cluj-Napoca
  - Centrul de Medicina Genomica, Timisoara
- Colaborare cu:
  - Centrul Regional de Genetica Medicala, Dolj
- Spitalul Robert Debre (Dr AC Tabet) si Spitalul Bicetre (Dr J Bouligand), Paris, Franta
- Tehnici utilizate
  - Citogenetica clasica
  - FISH
  - PCR si variante
  - Stripassay
  - MLPA
  - SNP array/CGH array
  - NGS

1. TESTAREA GENETICA PATOLOGIA ENDOCRINA

- Anomalii de dezvoltare sexuala

NEONATAL

- OGE ANORMALE
  - Hipertrofie clitoridiana izolata
  - Hipospadias posterior izolat
  - Criptorhidie/ectopie testiculara unilaterala+micropenis
  - Criptorhidie/ectopie testiculara unilaterala+hipospadias
  - Criptorhidie/ectopie bilaterala: gonade palpabile in pozitie inghinala sau nici o gonada palpabila

1. TESTAREA GENETICA PATOLOGIA ENDOCRINA

Anomalii de dezvoltare sexuala

PUBERTAR

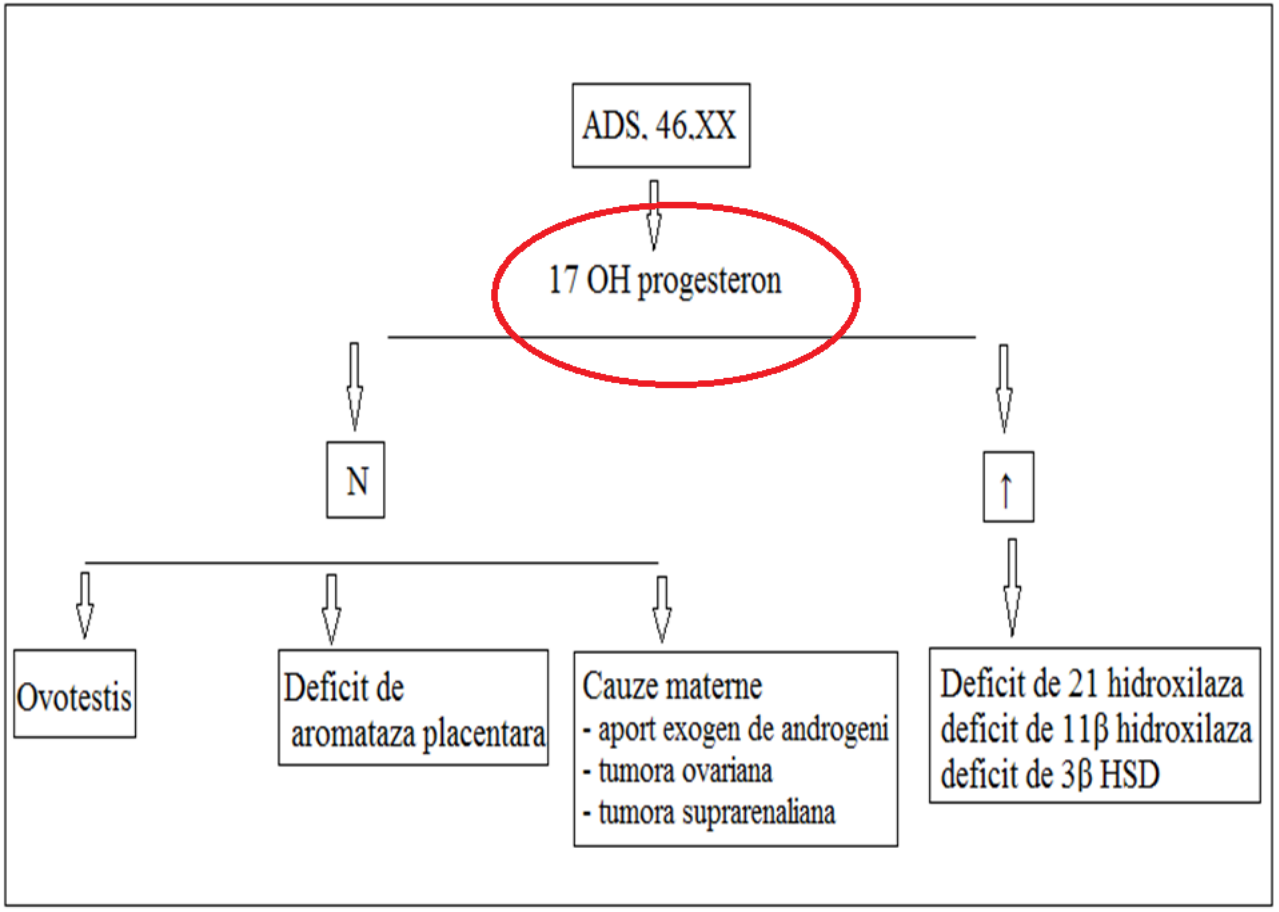
- **OGE ANORMALE**
  - Virilizare OGE la momentul reactivarii axului gonadotrop
    - Deficit 5 alfa reductaza, Deficit 17 cetoreductaza testiculara, ovotestis
- **RETARD PUBERTAR**
  - Disgenezie gonadala (Turner, Klinefelter)
  - Cariotip 45,X/46,XY
- **AMENOREE PRIMARA**
  - Rezistenta completa la androgeni

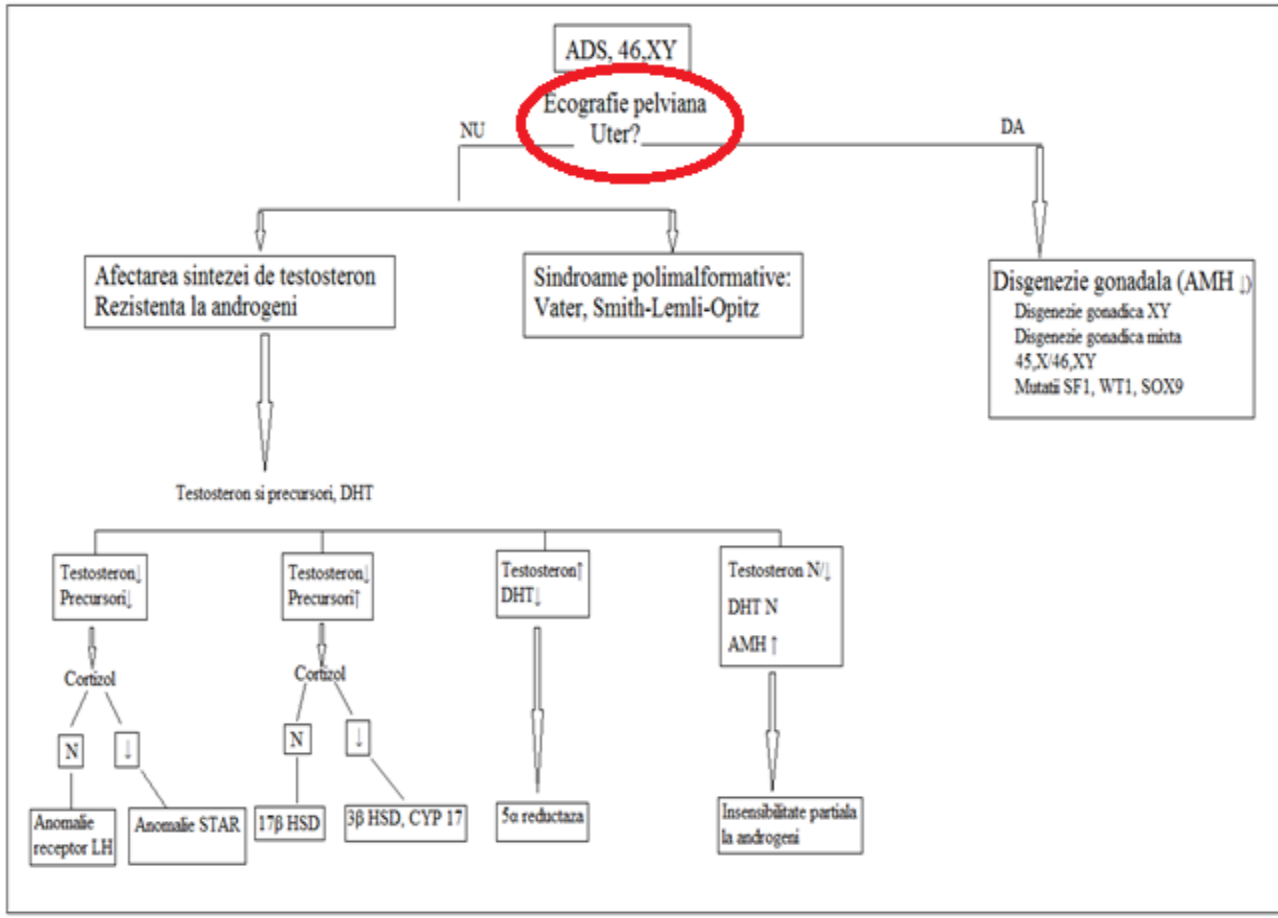
INVESTIGATII

- Dozari hormonale
  - 17OH progesteron
  - DHEAS
  - delta4 androstendion
  - Testosteron
  - AMH

- Ecografie gonade+OGI

- **Cariotip+SRY**
- Testare deficit 21OH
- Panel gene ADS


ADS. 46.XX
17 OH progesteron
N
↑
Ovotestis
Deficit de aromataza placentara
Cauze materne
- aport exogen de androgeni
- tumora ovariana
- tumora suprarenaliana
Deficit de 21 hidroxilaza
deficit de 11β hidroxilaza
deficit de 3β HSD


ADS, 46,XY
Ecografie pelviana
Uter?
NU
DA
Afectarea sintezei de testosteron
Rezistenta la androgeni
Sindroame polimalformative:
Vater, Smith-Lemli-Opitz
Disgenezie gonadala (AMH ↓)
Disgenezie gonadica XY
Disgenezie gonadica mixta
45,X/46,XY
Mutatii SF1, WT1, SOX9
Testosteron si precursori, DHT
Testosteron↓ Precursori↓
Testosteron↓ Precursori↑
Testosteron↑ DHT↓
Testosteron N/↓
DHT N
AMH ↑
Cortizol
Cortizol
N
↓
N
↓
Anomalie receptor LH
Anomalie STAR
17β HSD
3β HSD, CYP 17
5α reductaza
Insensibilitate partiala la androgeni

ADS, IN PRACTICA

- Cariotip+SRY
- Deficit 21 hidroxilaza – stripassay 11 mutatii mai frecvente
- Panel gene:
  - TruSight One 4800 gene OMIM morbide

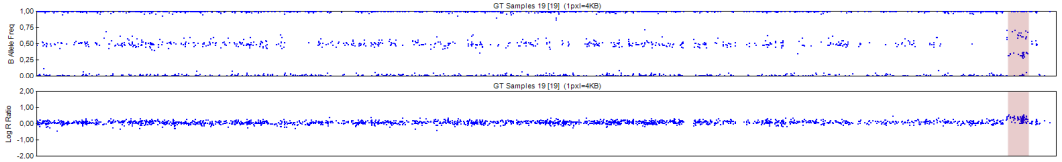
Patient	age(yrs)	social gender	phenotype	Caryotype	Hormonal investigation	Ultrasounds	SNP array	NGS
p1	10	male	Micropenis. 5th clinodactyly	46,XY	Testo N DHT ↓	N gonads, no mullerian derivates	no pathogen CNV	N
p2	18	female	clitoridian hypertrophy	46,XX	N	gonads, OGI VN	no pathogen CNV	N
p3	1	female	Right inguinal hernia. Feminin OGE. 2 aunts maternal line with primary amenhoree	46,XY	N	left gonads in inguinal conduct. Absence muller residus	no pathogen CNV	AR
p4	5	male	Penoscrotal hipospadias. Craniofacial dysmorphism. Blefarophimosis. Short stature. Aortic bicuspidia. DSV. Development delay	46,XY	N	gonads N, no mullerian residus	VOUS CNV SYCE1	N
p5	7	male	Micropenis. Testicular hypoplasia	46,XY	N		VOUS CNV SYCE1	
p6	18	male	Micropenis. Pubertary delay	46,XY	N		CNV dup 16p11.2	
p7	1	female	clitoridian hypertrophy	46,XX	17OHP↑	uterus	Del CYP21A2	
			penoclitoridian gland, labial hypertrophy					

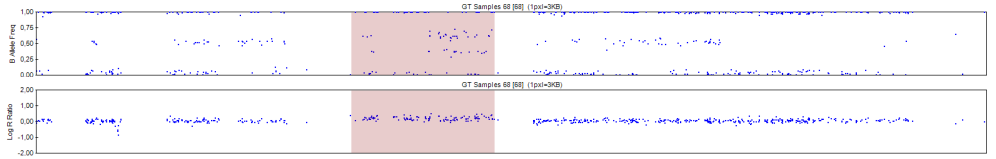
Patient	age(yrs)	social gender	phenotype	Caryotype	Hormonal investigation	Ultrasounds	SNP array	NGS
p9	3	male	micropenis. Hipospadias	46,XY	N	no muller residus	no pathogen CNV	
p10	20	female	amenoree. Hirsutism. Gonadal dysgenesis	46,XX	N		no pathogen CNV	
p11	1	female	clitoris hypertrophy, labial hypertrophy, vaginal and uretral meatus presents, right inguinal hernia	46,XY	T DHT ↑	in inguinal hernia- ovalary tumors with testicule appearance	no pathogen CNV	AR
p12	2	male	proximal hypospadias. Criptorchidism	46,XY			no pathogen CNV	
p13	3	female	gonadal dysgenesis, craniofacial dysmorphisms, short 4th and 5th metacarpals, 5th clinodactyly, SGA, short stature	46,XY	AMH, testo↓	no muller residus	no pathogen CNV	
p14	3	male	severe hypospadias	46,XY	N		no pathogen CNV	MAMLD1
p15	14	male	Penian hypospadias. Right cryptorchidism. Ginecomastia. Pubertal development.	46,XY	N	no muller residus, left testicule in inguinal conduct, a smaller tumor with the same localisation in right side	no pathogen CNV	
p16	3	male	Bilateral cryptorchidism. Gonadal dysgenesis	46,XY	LH,FSH ↑↑↑	left scleroatrophic testicule, ecografic, absent right testicule	no pathogen CNV	
			penoscrotal					

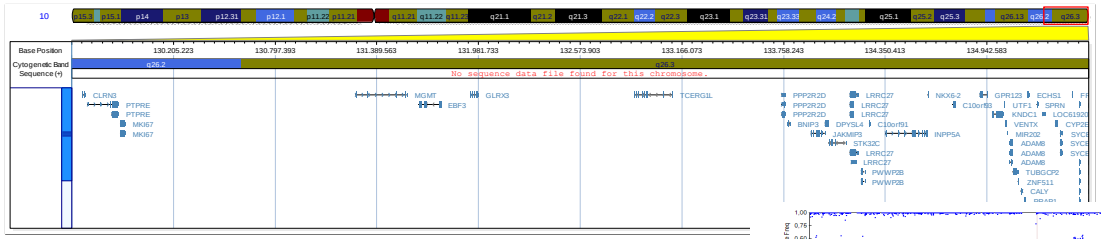
REZULTATE SNP ARRAY

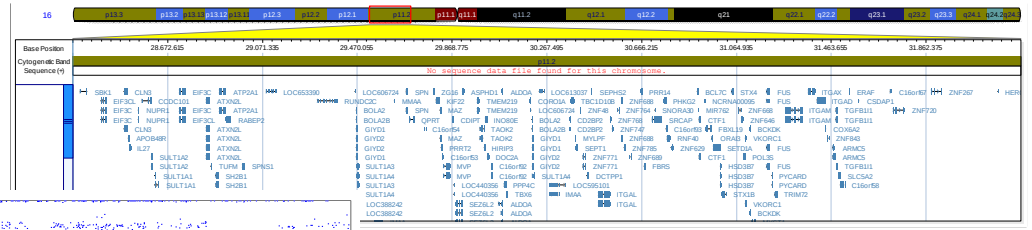
17 patients – 3 CNV VOUS/pathogenic

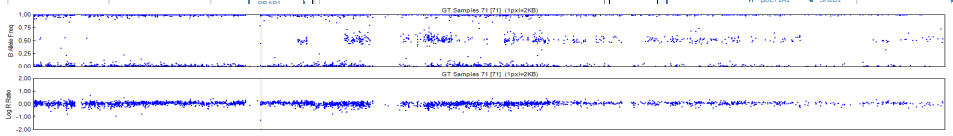
Patient	chr	start	stop	size	CNV	Gene	Interpretation
4	10	135257091	135378802	121711	Dup	SYCE1	VOUS
5	10	135252347	135378802	126455	Dup	SYCE1	VOUS
6	16	29595483	30192561	597078	Dup	16p11.2	Pathogenic
7	6	32005904	32006896	992	Homo del	CYP21A2	Pathogenic

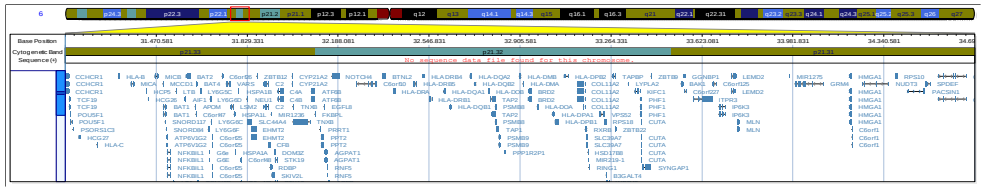












NGS RESULTS

- 9 tested patients

- 6 patients
- MAMLD1 (Xq28): c.1066C>T exonic stopgain mutation
  - Xlr
  - Clinical picture – micropenis, hypospadias, bifid scrotum

  - UMD predictor: pathogenic
  - EXAC: 2 alleles for 121410 chromosomes

- AR gene - 2 patients

RETARDUL PUBERTAR

I. ***Forma centrala***

Hipogonadism hipogonadotrop:

- *tranzitor*
- *permanent*

PANEL GENE

II. ***Forma periferica***

Hipogonadism hipergonadotrop:

- afectare gonadala primara – intotdeauna patologic
- cel mai frecvent disgenezie gonadala secundara anomaliilor cromozomilor sexuali

CARIOTIP

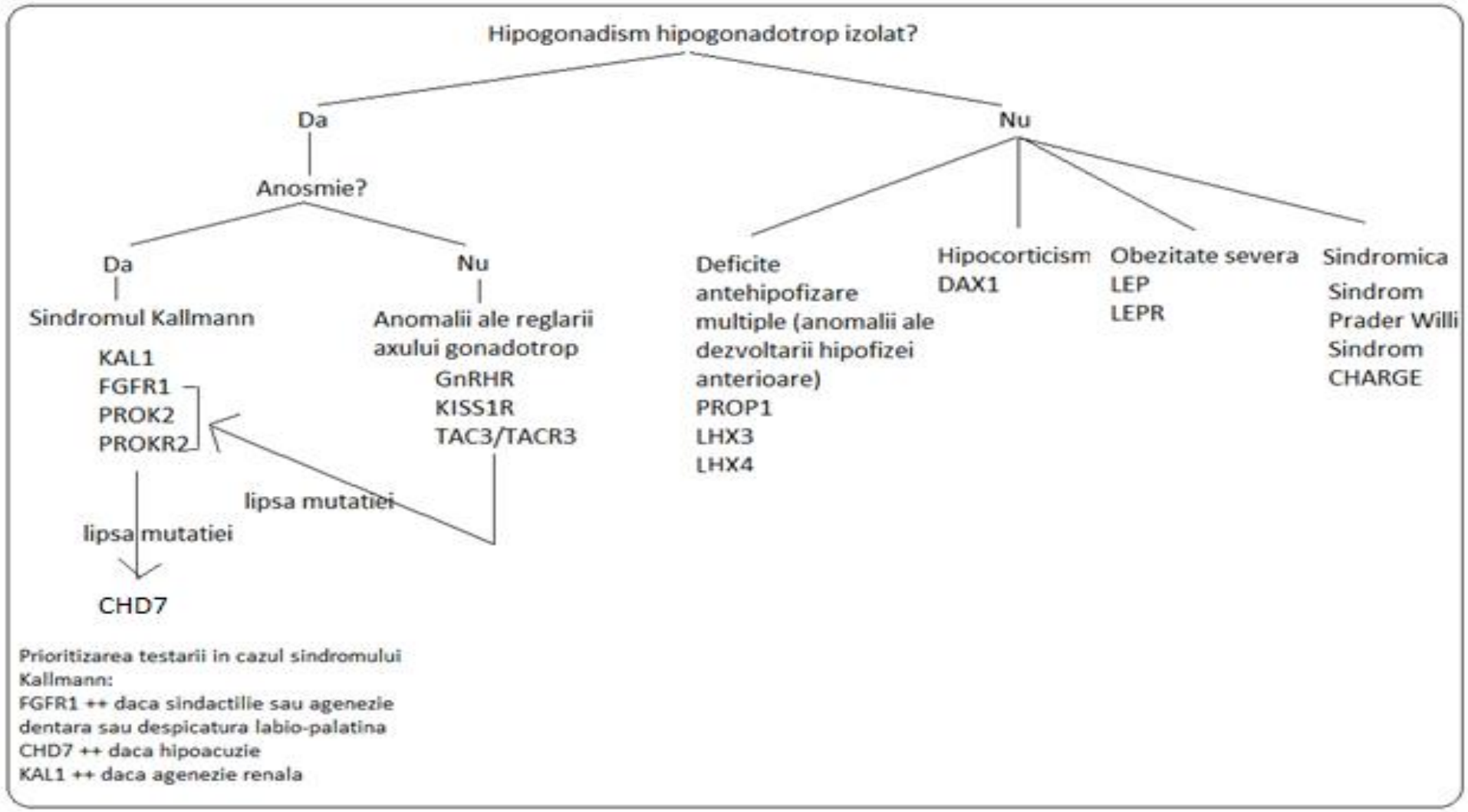
RETARDUL PUBERTAR

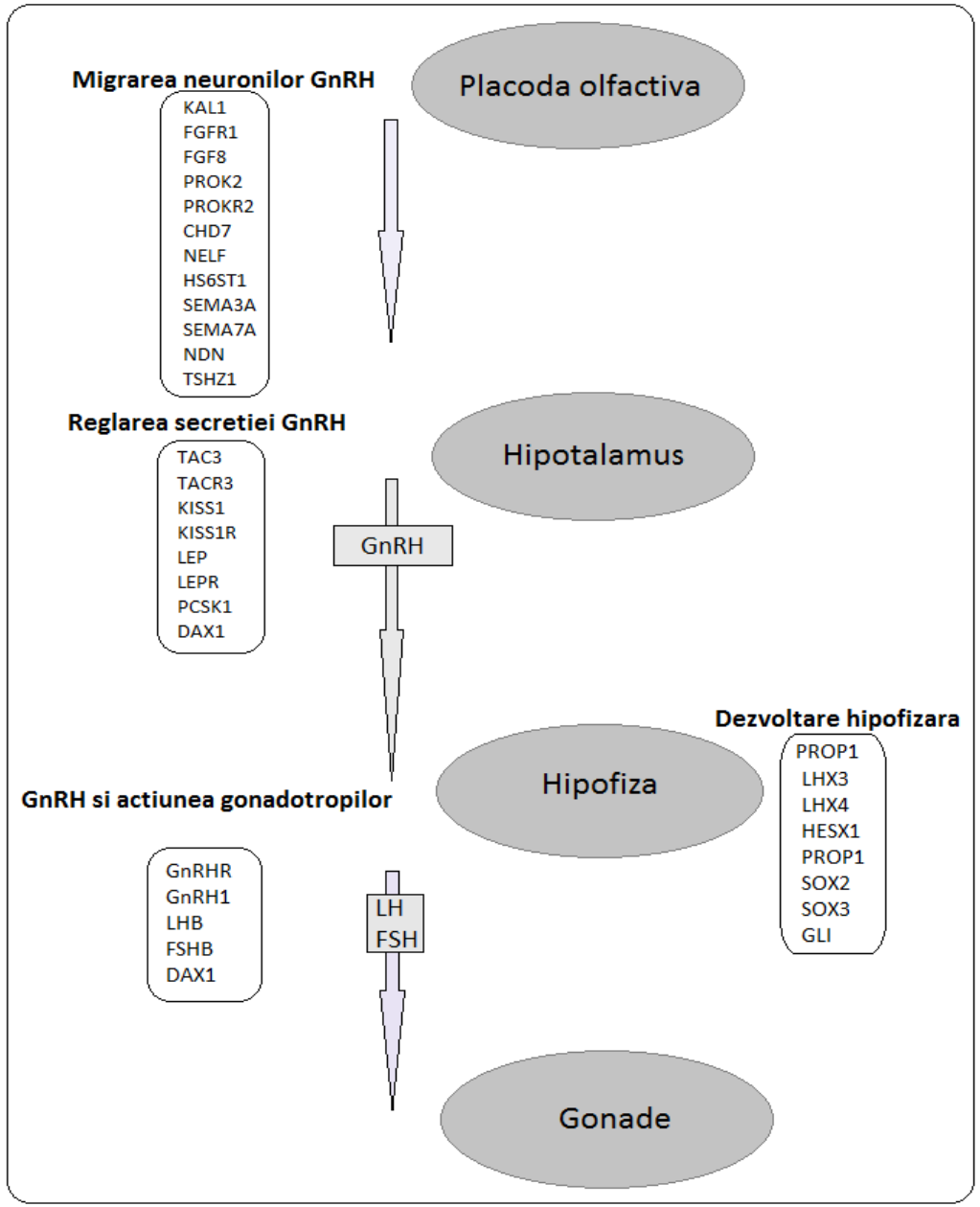
HIPOGONADISMUL HIPOGONADOTROP CONGENITAL

- *Suspiciune clinica daca*
  - micropenis si/sau criptorhidism la nou-nascutii de sex masculin
  - retardul pubertar la o varsta osoasa mai mare de 13 ani

- *izolat* (este afectat doar axul gonadotrop)
  - 50% din cazuri cu anosmie/hipoosmie = *sindrom Kallmann*
- *asociat* altor afectari endocrine

- *non-sindromic*
- *sindromic* anomalii ale liniei mediane -despicaturi labio-palatine, agenezii dentare- anomalii ale urechii si surditate, agenezii renale, malformatii cardiace, anomalii scheletice ale extremitatilor, sinkinezii bimanuale


Hipogonadism hipogonadotrop izolat?
Da
Nu
Anosmie?
Da
Nu
Sindromul Kallmann
KAL1
FGFR1
PROK2
PROKR2
Anomalii ale reglarii axului gonadotrop
GnRHR
KISS1R
TAC3/TACR3
Deficite antehipofizare multiple (anomalii ale dezvoltarii hipofizei anterioare)
PROP1
LHX3
LHX4
Hipocorticism
DAX1
Obezitate severa
LEP
LEPR
Sindromica
Sindrom Prader Willi
Sindrom CHARGE
lipsa mutatiei
lipsa mutatiei
CHD7
Prioritizarea testarii in cazul sindromului Kallmann:
FGFR1 ++ daca sindactilie sau agenezie dentara sau despicatura labio-palatina
CHD7 ++ daca hipoacuzie
KAL1 ++ daca agenezie renala


Migrarea neuronilor GnRH
KAL1
FGFR1
FGF8
PROK2
PROKR2
CHD7
NELF
HS6ST1
SEMA3A
SEMA7A
NDN
TSHZ1
Placoda olfactiva
Reglarea secretiei GnRH
TAC3
TACR3
KISS1
KISS1R
LEP
LEPR
PCSK1
DAX1
Hipotalamus
GnRH
Dezvoltare hipofizara
PROP1
LHX3
LHX4
HESX1
PROP1
SOX2
SOX3
GLI
Hipofiza
GnRH si actiunea gonadotropilor
GnRHR
GnRH1
LHB
FSHB
DAX1
LH
FSH
Gonade

- HHC izolat – NGS – 9 pacienti
  - Panel gene: GNRHR, GNRH1, KISS1R, KISS1, TACR3, TAC3, KAL1, FGFR1, FGF8, PROKR2, PROK2, WDR11, CHD7, SEMA3A, NSMF, HS6ST1, FSHB, LHB, SOX3, FGF17, IL17RD, DUSP6, SPRY4, FLRT3, PROP1, NR0B1, PCSK1, LHX4, HESX1, OTX2, RNF216, OTUD4, SOX2, POU1F1, SOX10, KALP, CUL4A, CUL4B, GNRH2, NRP1, NRP2, SIX6, PDYN, OPRK1, TAC1, TACR1, TACR2, NPVF, NPFFR1, PLXNA1, SEMA7A, LHX3, NPY, LHX2, POU2F1, POU3F2, SLIT2, ROBO3, LEPR, LEP, SLIT3, CGA, INHBA, PRLR, PCSK2, PLXNC1, DCC, ZIC1, LIFR, FARP2
- HHC+obezitate – MS-MLPA Prader Willi

INSUFICIENTA OVARIANA PREMATURA

- Cariotip
- Detectie premutatii FMR1

TESTARE GENETICA IN OBEZITATE

OBEZITATE

+

- Hipotonie+tulburari de alimentatie la nou-nascut
- Hipostatura, anomalii scheletale (brahimetatarsie)
- Endocrinopatii: hipogonadism
- Retard psihomotor
- Tulburare vizuala, auditiva, retard limbaj
- Epilepsie, tulburare de comportament
- Sindrom dismorfic, sindrom malformativ

=>OBEZITATE GENETICA

OBEZITATEA SINDROMICA IN PRACTICA

- Prader Willi: MS-MLPA - deletii si UPD
- Obezitate+Retard mental: PCRq - CNV 16p11.2; 15q11.1
- Obezitate+retard mental+sindrom dismorfic+sindrom malformativ: MLPA microdeletii
- SNP array/CGH array – ideal!

HIPOSTATURA

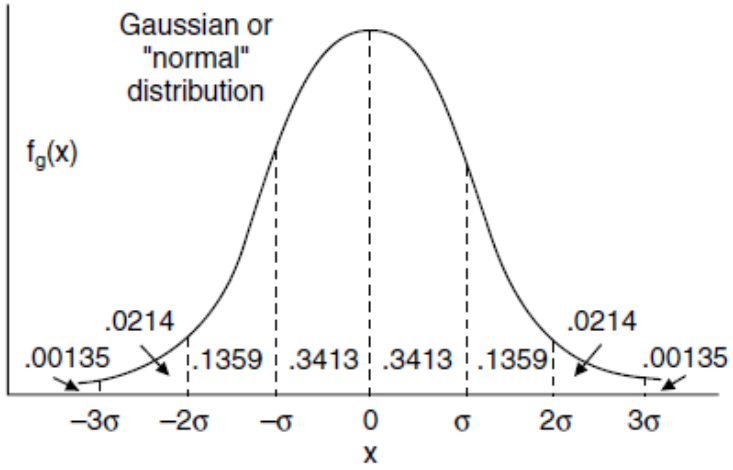
Cresterea - Proces multifactorial

Factori genetici > 80%

- Factori etnici, talia parentala

Factori de mediu

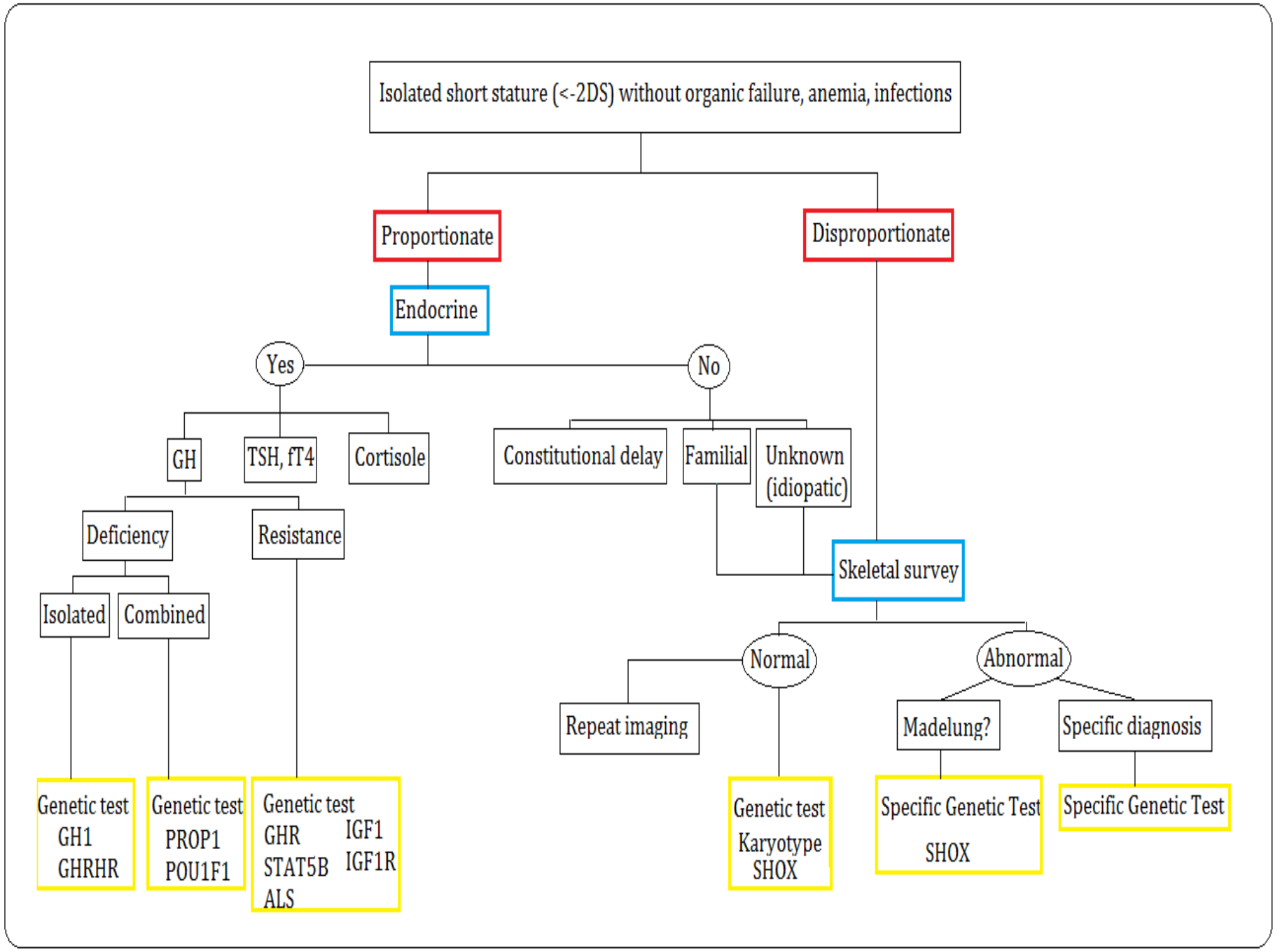
- Factori de mediu!
  - Ultimii 150 ani – trend secular



HIPOSTATURA - CLASIFICARE

- primara: afectiuni intrinseci ale osului
  - *Displazii scheletale (3%)*
  - *Sindroame genetice (21%)*
  - *RCIU fara recuperare (12%)*
- secundara: afectiuni ce modifica fiziologia cartilajului de crestere
  - *Endocrine (11%)*
  - *Afectiuni cronice (3%)*
  - *Malnutritie, cauze metabolice, psihosociale*
- Idiopatica (50%)
  - *Hipostatura Constitutionala (idiopatica), familiala*

Lam WFF, et al., Chin Med J 2002:115, 607-6117



ACMG practice guideline: Genetic evaluation of short stature(Seaver LH and Irons M, 2009)

HIPOSTATURA IZOLATA IN PRACTICA

- La fete – cromozomi sexuali si SHOX - MLPA
- La baieti – 45,X/46,XY? – cariotip

Afectare endocrina – Hipostatura proportionata

- PROP1
- Ideal Panel gene

Afectare scheletala – Hipostatura disproportionata

- SHOX - MLPA
- FGFR3 - PCR
- Ideal panel de gene

Tableau I . Proportion d'anomalies osseuses chez les patients ayant un retard statural sans étiologie retrouvée

	CAS	Radios (nb)	Radios (%)	N	An	MOC/ R (%)	MOC/ T (%)
RCIUN	35	9	25,7%	8	1	11,1%	2,9%
RCIUNR	119	45	37,8%	37	8	17,8%	6,7%
RCIUP	45	23	**51,1%**	10	13	**56,5%**	28,9%
Total RCIU	**199**	**77**	**38,7%**	**55**	**22**	**28,6%**	**11,1%**
ISS	243	140	57,6%	84	56	**40%**	23%
ISSP	80	45	56,2%	32	13	28,9%	16,3%
Total ISS	**323**	**185**	**57,3%**	**116**	**69**	**37,3%**	**21,4%**
TOTAL RCIU+ ISS	**522**	**262**	**50,2%**	**171**	**91**	**34,7%**	**17,4%**

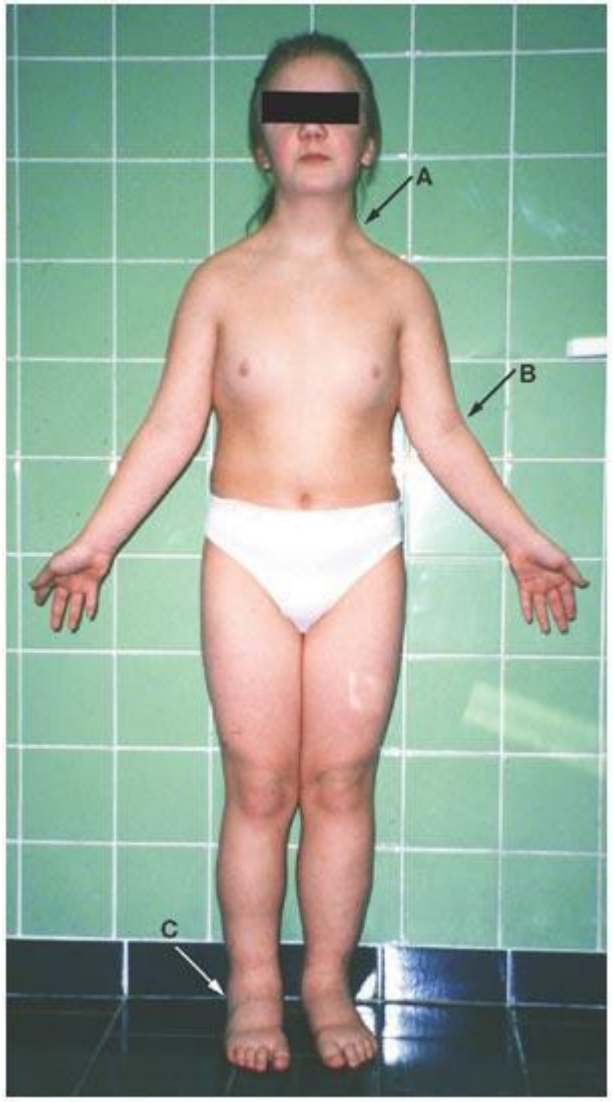
RCIUN : RCIU avec rattrapage de la croissance, RCIUNR : RCIU sans rattrapage de la croissance, RCIUP : RCIU dont un parent a une taille <-2DS, ISSN : ISS avec parents de taille normale, ISSP : ISS avec un parent de taille <-2DS, Radio (nb) : radiographies réalisées en valeurs absolue, Radio (%) : pourcentage de radiographies réalisées. N : radiographies normales, An : Anomalie osseuse, MOC/R : pourcentage d'anomalies osseuses sur le nombre de radiographies réalisées, MOC/T : pourcentage d'anomalies osseuses sur la totalité du groupe.

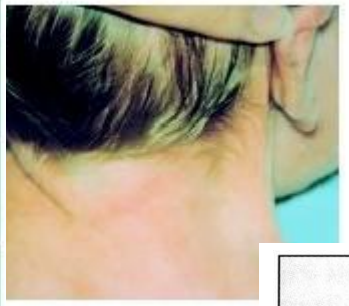
HIPOSTATURA SINDROMICA IN PRACTICA

- Daca exista suspiciune clinica
  - test genetic tintit

- Daca nu exista suspiciune clinica
  - Evaluare genomica: MLPA, SNP array, NGS – panel de gene, exom

SINDROM TURNER





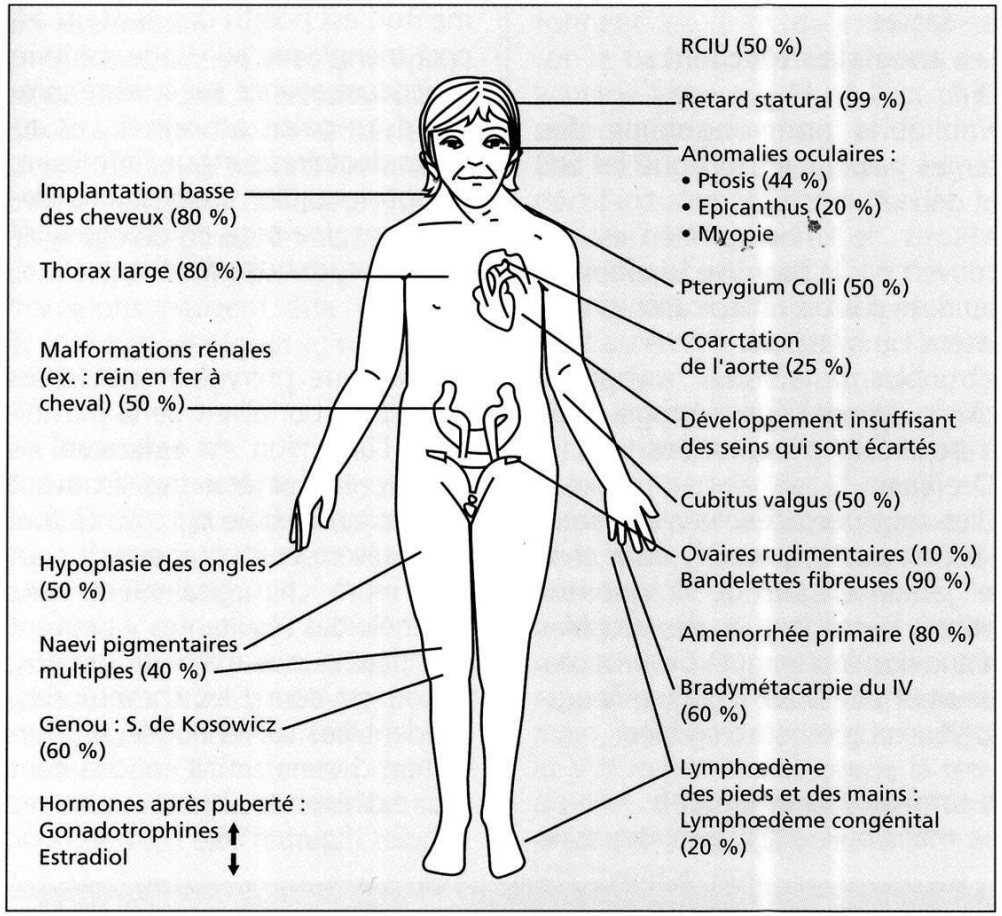
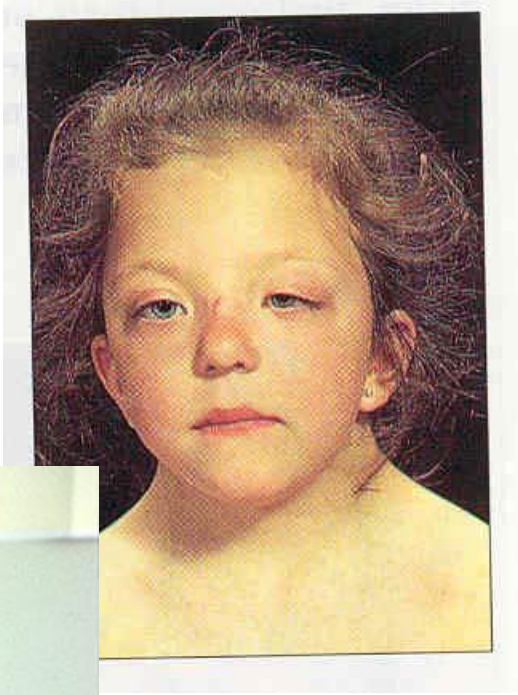
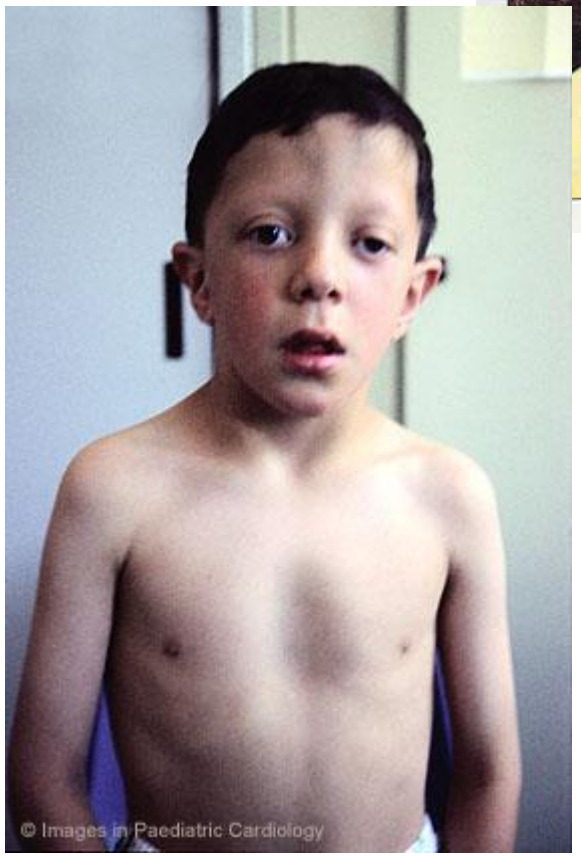


Figure n° 5 : schéma synthétique. Principales dysmorphies et malformations dans le syndrome de

SINDROM NOONAN

- PTPN11

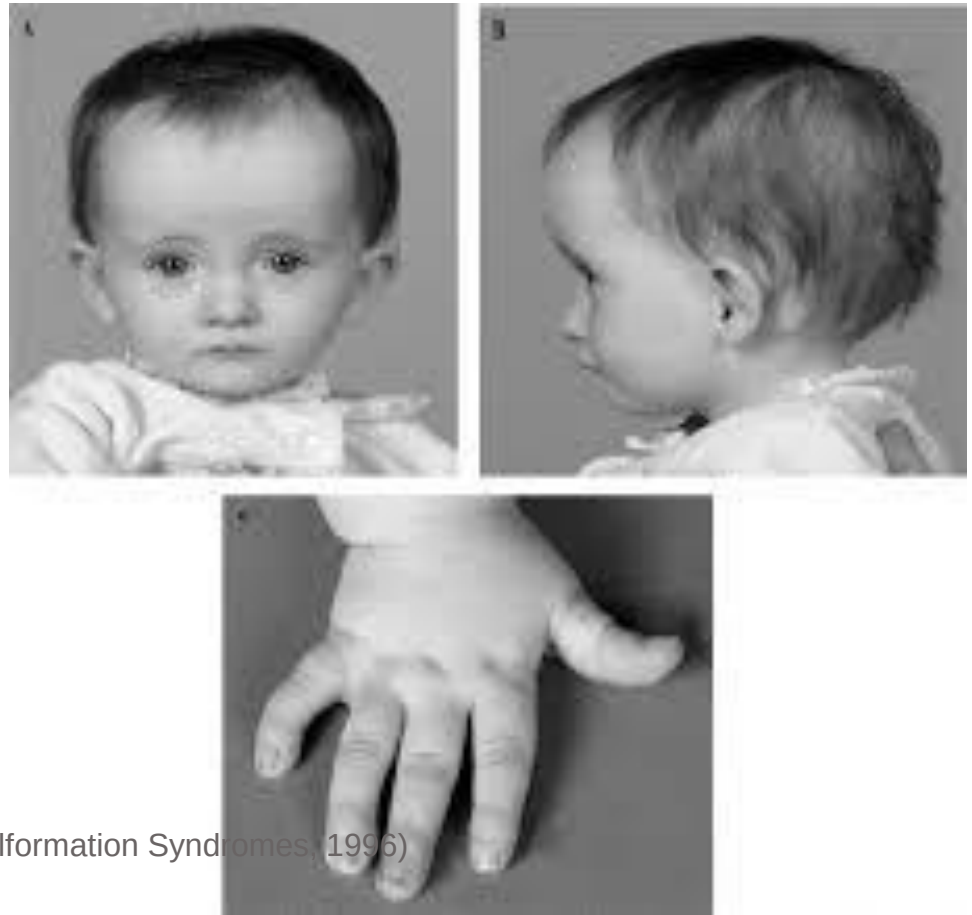




from, Baraitser and Winter, Color Atlas of Congenital Malformation Syndromes, 1996

SINDROM RUSSELL-SILVER

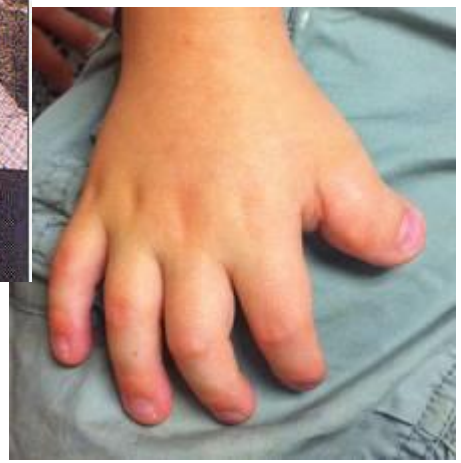
- 10% - UPD 7mat
- Anomalii ale metilarii 11p15.5-35%



(from, Baraitser and Winter, Color Atlas of Congenital Malformation Syndromes, 1996)

SINDROM RUBINSTEIN-TAYBI

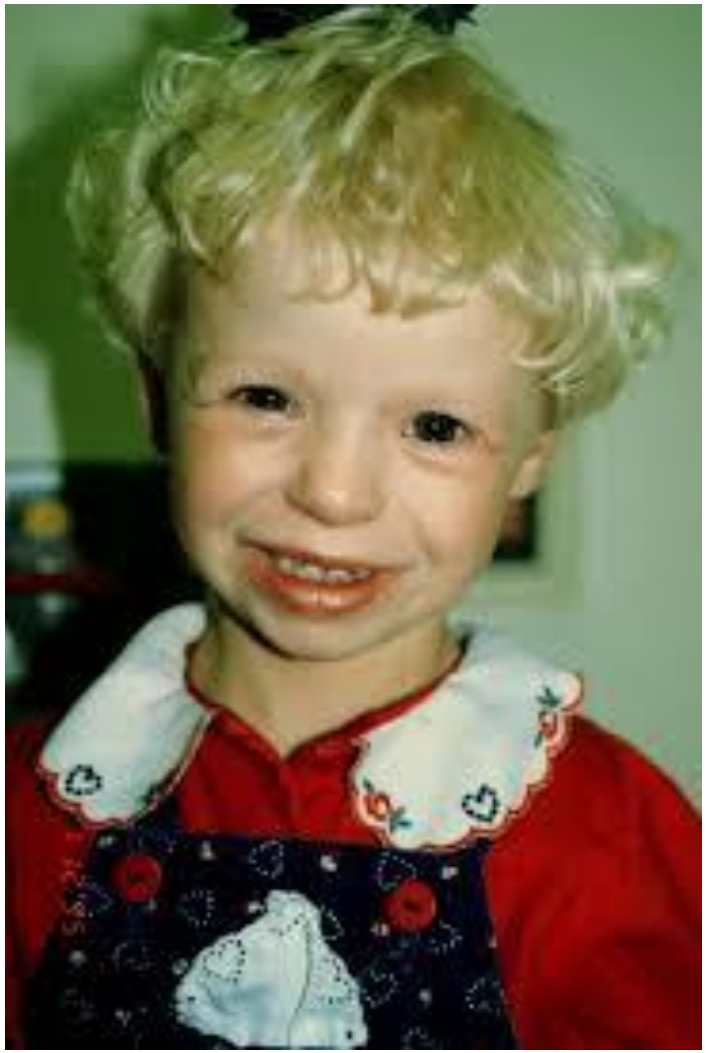
- <10% del 16p13.3
- 10-20% del/dup CREBBP



(from, Baraitser and Winter, Color Atlas of Congenital Malformation Syndromes, 1996)

SINDROM WILLIAMS

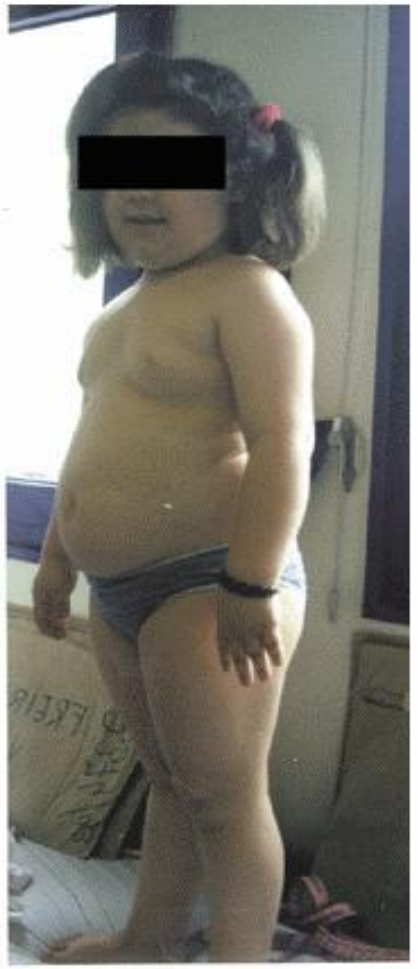
- 99%: del 7q11.23



(from, Baraitser and Winter, Color Atlas of Congenital Malformation Syndromes, 1996)

PSEUDOHIPOPARATIROIDISM TIP IA

- GNAS1 anomalii ale metilarii



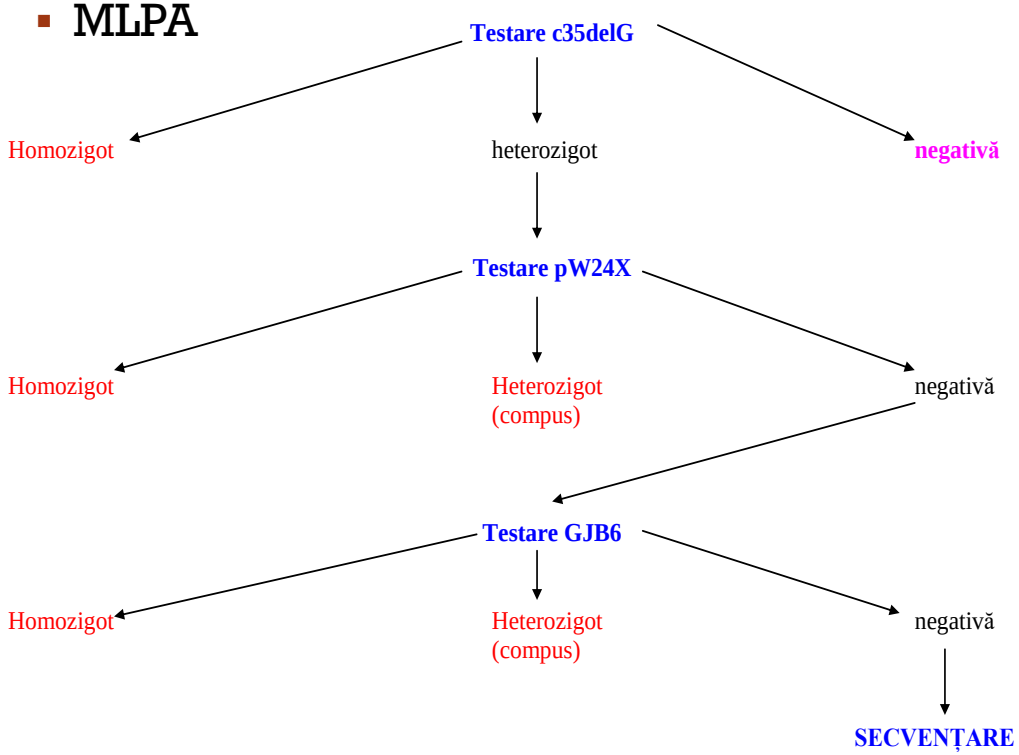
2. TESTAREA GENETICA

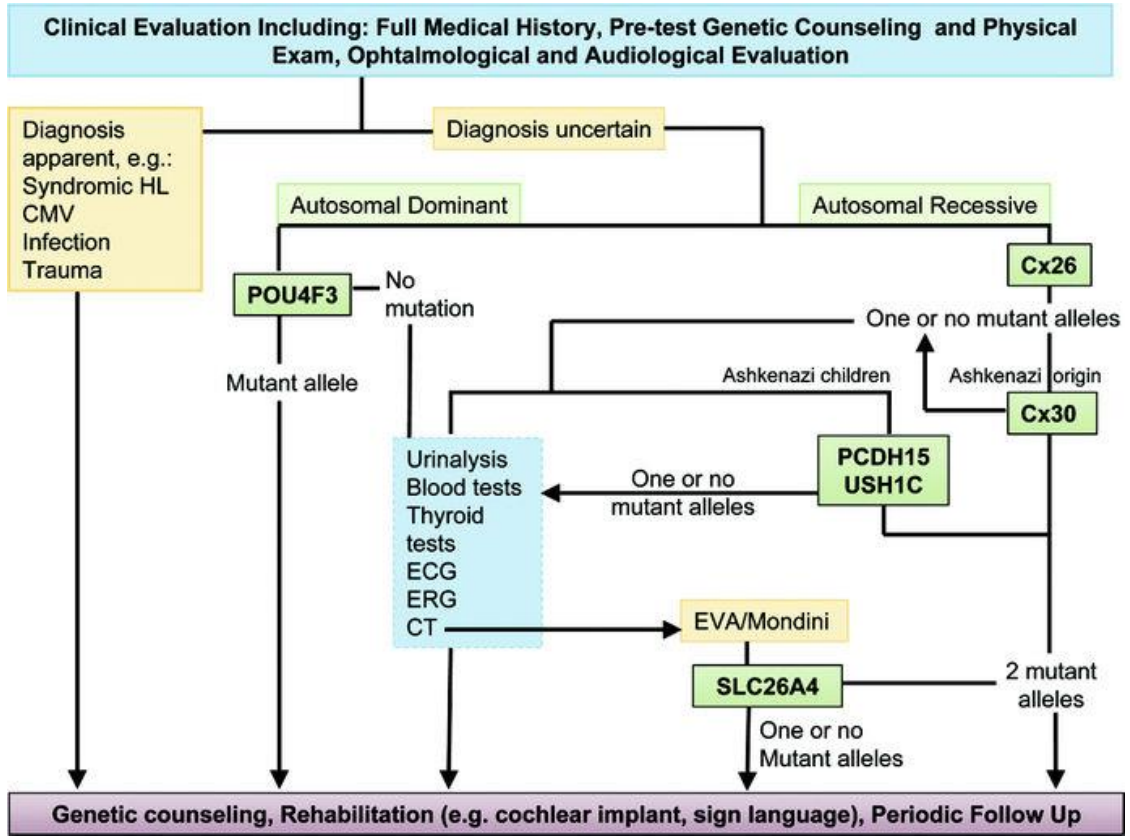
- FIBROZA CHISTICA
- BOLI GENETICE DE METABOLISM

  Boala Gaucher detectia a 8 mutatii comune GBA prin metoda StripAssay
  Deficit de alfa 1 antitripsina A1AT – identificarea alelelor M,S si Z

3. PATOLOGIA SENZORIALA

- SURDITATEA nonsindromica – 328 cazuri analizate
  - GJB2 - 35delG, W24X
  - GJB6 - D13S1830 si D13S1854
  - MLPA





Lazar C, Int J Pediatr Otorhinolaryngol, 2010

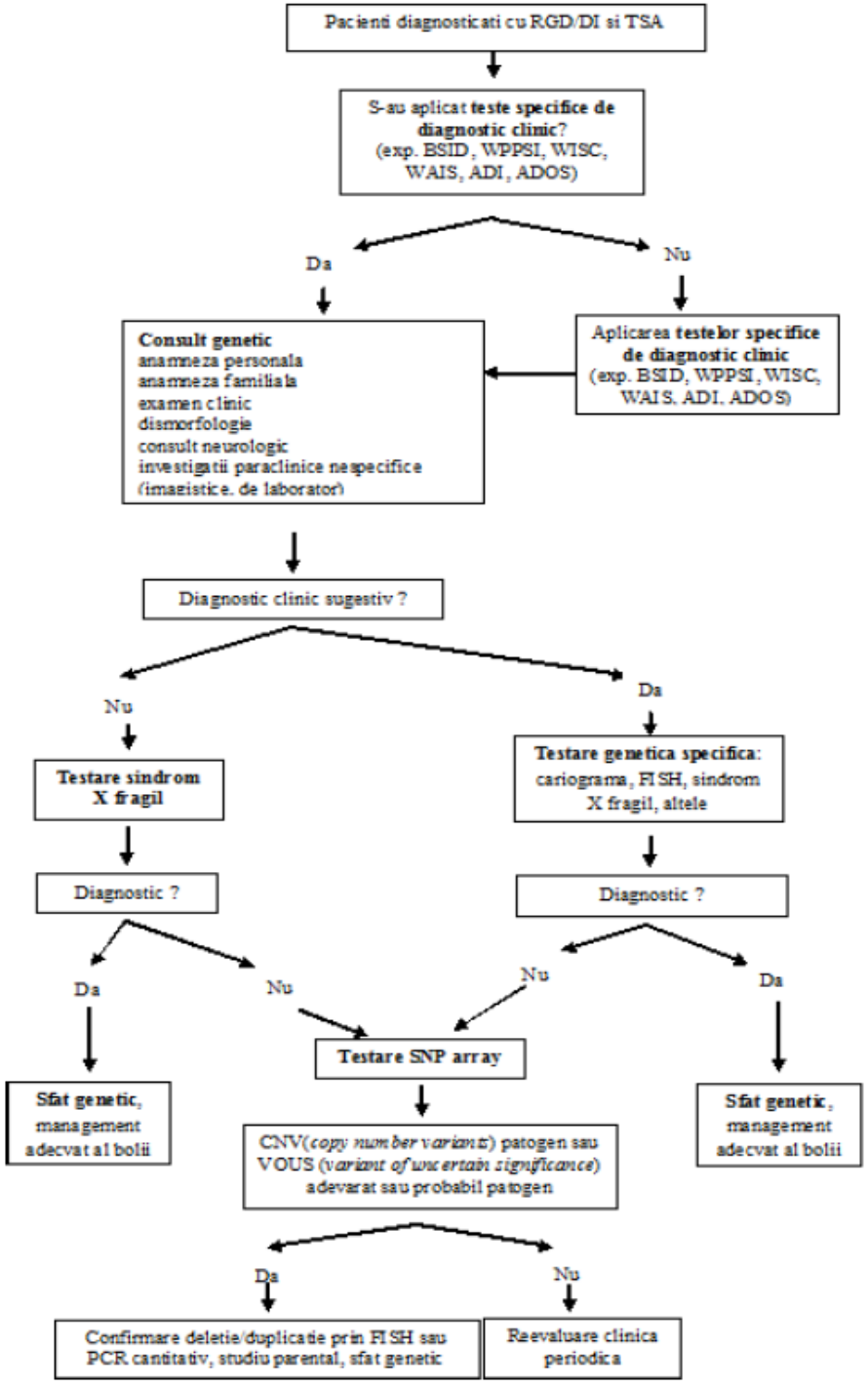
3. PATOLOGIA NEUROPSIHIATRICA

-X fragil

-cariotip (1200 cazuri analizate)

-MLPA microdeletii (430 cazuri analizate)

-SNP array/CGH array (210 cazuri analizate)



CONCLUZII

DIAGNOSTIC MAI BUN

- un randament diagnostic bun şi astfel un bun raport cost-beneficiu derivă din **indicaţia adecvată a acestor teste** – **centre de expertiza** cu echipa multidisciplinara
  - Cluj – Deficit 21 hidroxilaza, Boli lizozomale
  - utilizarea ghidurilor pentru testare genetică!
- testarea genetica presupune din ce in ce mai mult folosirea unor **tehnologii de analiza a genomului, utilizate deja la noi in tara – pentru o interpretare buna – trebuie un numar limitat de patologii/centru**

TRATAMENT MAI BUN

- **intelegerea unui mecanism molecular va conduce la alegerea unei terapii de precizie** (ex.tulburarile din spectrul autist, oncologie)