Is a fetal pathology examination still necessary after prenatal exome sequencing ?

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Fetal structural anomalies

3% — Major, Minor

Various etiologies

Fetal Medicine — Impact, Counseling — Type, Number, Severity, Cognitif impairement ?, Cause ?

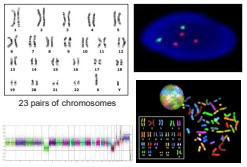
Prognosis

➢Management

- Reassurance
- Prenatal treatment
- Neonatal care
- Support
- Termination of pregnancy

Genetic investigations from Karyotype to chromosomal microarray

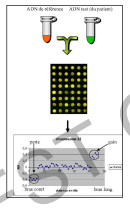
- **Structure, CNV**
  - Karyotype: Mb-scale
  - CNVs and balanced chromosomal abnormalities
  - FISH



15 %

CMA

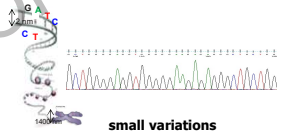
Submicroscopic CNVs



≈5%

Genetic investigations From Sanger sequencing……to NGS

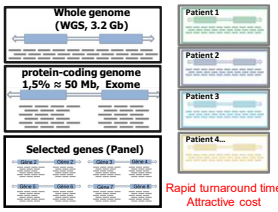
- **Conventionnel Sanger sequencing**
  - Few hundred base pairs
  - Individual genes



small variations

SNVs

- **NGS (or high throughput sequencing)**
  - Millions of DNA fragments
  - Several patients simultaneously



Rapid turnaround time
Attractive cost

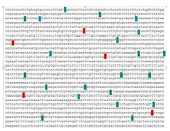
Genetic variants: a continuum

SNV		CNV
cgcgtatcagagagatcctcgc	cgcgtatcagagagatcctcgc	cgcgtatcagagagatcctcgc
tagagctcgcgcgagctcgctg	tagagctctcgcgagctcgctg	ta----------
agggcgcccctctctagaaaag	agggcgcccctctctagaaaag	----------
agagctccgctagagagatctc	agagctccgctagagagatctc	----------
gctgatcgatgctagctagtcg	gctgatcgatgctagctagtcg	----------
atcgatcgatcgatcgatcccc	atcgatcgatcgatcgatcccc	gctctctatat
cgcgccgcgcgcgcgggggga	cgcgccgcgcgcgcgggggga	
gaaagctctctataattatc	gaaagctctctataattatc	

- Any **type** (base change, add, loss, duplication, inversion …)
- Any **size** (one base to whole chromosome)
- Different **implications**

➢ Without effect
➢ Beneficial effect
➢ Confere susceptibility
➢ Disease causing

The genome is variable



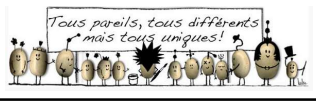
cgcgtatcagagagatcctcgcta--

---
gctctctatataattatctcgctaatacgt
gatcgtcagtcgtacg

≈ 1 variation every 1 000 bases

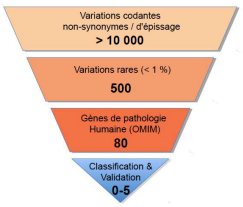
Between 2 individuals
0.1 % of differences

At the genome level
➢ 1 000 structural variants (CNV)
➢ 3 to 4 millions small variations (SNV)
300 « deleterious »
50-80 reported in diseases



Variants prioritization and classification

Priorisation des variations



5	Pathogenic	Previously reported as disease causing
4	Likely pathogenic	Not previously reported , but type/nature predicts its pathogenicity
3	Unknown significance	Insufficient evidence to classify it at this time - could be pathogenic or benign ➢ Needs further analysis ➢ Classification may vary with time ➢ **Can not be used for counseling / prenatal diagnosis**
2	Likely benign	Not reported, not expected to be disease causing
1	Benign	Known not to cause a disease

Richards et al., Genetics in medecine, 2015 (ACMG)

ACMG STATEMENT | Genetics inMedicine

The use of fetal exome sequencing in prenatal diagnosis: a points to consider document of the American College of Medical Genetics and Genomics (ACMG)

Kristin G. Monaghan, PhD[1], Natalia T. Leach, PhD[2], Dawn Pekarek, MD[3], Priya Prasad, MD[4] and Nancy C. Rose, MD[5]; on behalf of the ACMG Professional Practice and Guidelines Committee

Known disease genes

- It is recommended that laboratories offering prenatal ES report pathogenic and likely pathogenic variants, as determined using ACMG variant interpretation guidelines in known disease genes consistent with the reported fetal phenotype.[18,21]

VUS not reported in prenatal setting

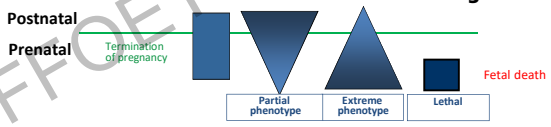
Fetal WES: diagnostic rate and VUS

	Number	Diagnostics	VUS
Retrospective			
Alamillo et al. 2015	7	3 (47%)	1 (14%)
Vora et al. 2017	15	7 (47%)	inclus
Carss et al., 2014	30	3 (10 %)	5 (17%)
Shamseldin et al. 2018	44	22 (50%)	15 (34%)
Yates et al. 2017	84	17 (20%)	7 (9%)
Fu et al. 2018	**196**	**47 (24%)**	**25 (13%)**
Prenatal settings			
Westerfield et al., 2015	10	3 (30%)	1 (10%)
Pangalos et al., 2016	14	5 (36%)	2 (14%)
Drury et al., 2015	24	5 (21%)	1 (4%)
Normand et al., 2018	146	46 (32%)	inclus
Petrovski et al., Lancet 2019	**234**	**24 (10%)**	**46 (20%)**
Lord et al., Lancet 2019	**596**	**52 (8 %)**	**24 (4 %)**

➢ **Diagnostic rate: 8,5-24 % (sytematic analysis , no selection)**

➢ **Variants of unknown significance: 4-20 %**

Caracterization of fetal disorders : challenges



- Potential difficulties in interpreting genetic variants → Variants of unknown significance (VUS)
- Many traits can not be assessed in utero → Not accessible to ultrasound / absent at this period of life – incomplete phenotypes
- Observed signs rarely specific of one disorder → Several or no hypothesis…
- Unknown prenatal signs → Better knowledge of prenatal signs of genetic disorders
- Different spectrum of severity → Clinical variability of genetic disorders, extreme phenotypes

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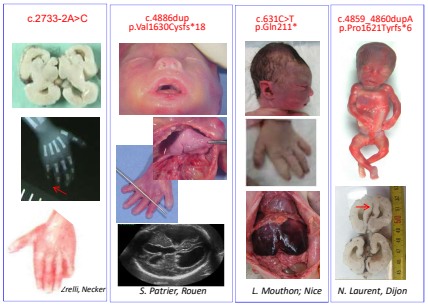
Spectrum of severity: Coffin Siris syndrome



Tsurusaki et al., 2012; 2014
Santen et al., 2013
Wieczorek et al., 2013

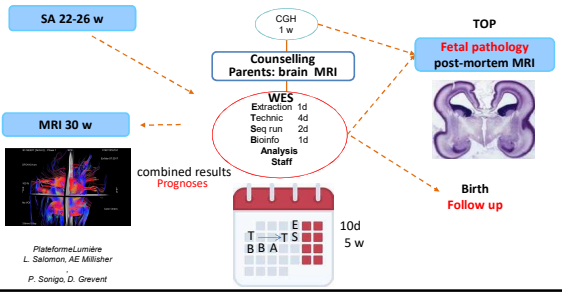
8 patients reported
Ietal
Somatic mosaicism

Dysmorphism
Cleft palate
Diaphragmatic hernia
Heart defects
○Hypoplastic left heart
○Atrio ventricular canal

ACC
Ventriculomegaly
Cortical anomalies
Nodular heterotopia

ARID1A (NM_006015.4)

WES in structural anomalies during pregnancy



3/45 ongoing pregnancies – outcome ?

Prenatal WES	Continuation of pregnancy	TOP
Molecular diagnosis : 15/45 (33%)	*7/42, 16%* DCC, DYNC2H1, OFD1, SLC26A3, COL1A1, ODAD4	*8/42 (19%)* - 5 FPE
No molecular diagnosis 30/45 (66%) including VSI: 5/45 (11%)	*22/42 (52 %)* Two diagnosis postnatally (GPC3, Silver-Russel) 4	*5/42* : **12%, - 4 FPE** *Genome sequenciing 4* 1

4/13 TOP, declined FPE

- A case without molecular diagnosis: brain anomalies -> no further analysis

- **A negative result doesn't exclude a genetic cause**
- Postnatal / postmortem phenotyping
- Need to reanalyse the negative data

Rapidely evolving knowledge in Genetics

10 % of diagnosis after reanalysis one year

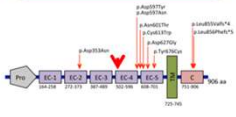
Prenatal exome sequencing	Continuation of pregnancy	TOP
3/48 ongoing pregnancies – outcome ?		
Molecular diagnosis : 15/45 (33%)	*7/42, 16%* DCC, DYNC2H1, OFD1, SLC26A3, COL1A1, ODAD4	*8/42 (19%)* - 5 FPE
No molecular diagnosis 30/45 (66%) including VSI: 5/45 (11%)	*22/42 (52 %)* Two diagnosis postnatally (GPC3, Silver-Russel) 4	*5/42* : **12%, - 4 FPE** *Genome sequenciing 4* 1

4/13 TOP, declined FPE

- A case without molecular diagnosis: brain anomalies -> no further analysis
- Signs not reported in the syndrome (hydramnios, *SKI*, HCN, *CREBBP*)
- Incomplete phenotype (isolated ACC, *CDH2*)

Incomplete phenotype

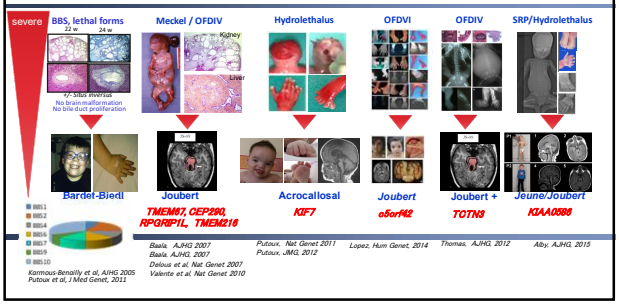
MCDA pregnancy
CCA in both female fetuses
Apparently isolated on ultrasound 24w
Parents: brain MRI normal
MCA: normal
WES: *CDH2* (N-cadherin , neuronal)
NM_001792.4: c.1522G>A,
p.(Glu508Lys) *de novo* (March 2020)
TOP



AJHG 2019 Oct 3;105(4):854-868.

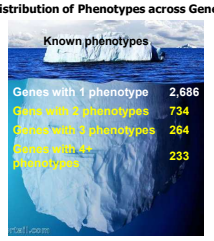
De Novo Pathogenic Variants in N-cadherin Cause a Syndromic Neurodevelopmental Disorder with Corpus Callosum, Axon, Cardiac, Ocular, and Genital Defects

Extreme phenotypes of ciliopathies



Phenotypes, OMIM statistics

Distribution of Phenotypes across Genes



2020

13/10/2021

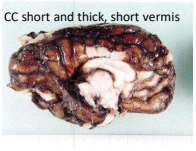
Genes with 1 phenotype	3,162
Genes with 2 phenotypes	820
Genes with 3 phenotypes	306
Genes with 4+ phenotypes	239

Reverse phenotyping

TOP at 34gw for brain and heart anomalies
Female
Facial dysmorphism
Overriding fingers
Kidney asymetry
Normal growth parameters

- weight : 2385 g
- Mensurations
  - VT : 47 cm
  - VC : 31 cm
  - HC : 31 cm (50e c)
  - feet : 6,8 cm

CC short and thick, short vermis



Smith-Lemli-Opitz syndrome

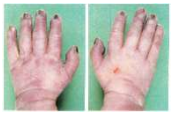
J. MARTINOVIC

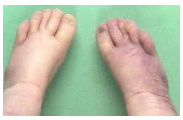
Disease definition

Smith-Lemli-Opitz syndrome (SLOS) is characterized by multiple congenital anomalies, intellectual deficit, and behavioral problems.

« callosome » panel : DHCR7
- **c.906C>G, p.(Phe302Leu) maternal**
- **c.385_412+5del, p.(Ile129Glyfs*3) paternal**

Reverse phenotyping





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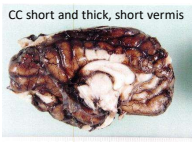


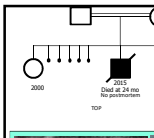
Brain weight 234 g (<5e p) -> Micrencephaly

Smith-Lemli-Opitz syndrome

J. MARTINOVIC

Atypical cases

« callosome » panel : DHCR7
- **c.906C>G, p.(Phe302Leu) maternal**
- **c.385_412+5del, p.(Ile129Glyfs*3) paternal**



2000

2015
Died at 24 mo
No postmortem
TOP

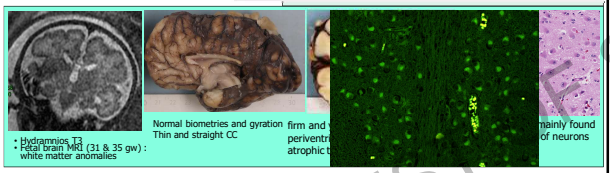
2016
36 SA

Neuronal ceroid lipofuscinosis

Suggest an update

Disease definition

Neuronal ceroid lipofuscinoses (NCLs) are a group of inherited progressive degenerative brain diseases characterized clinically by a decline of mental and other capacities, epilepsy, and vision loss through retinal degeneration, and histopathologically by intracellular accumulation of an autofluorescent material, ceroid lipofuscin, in the neuronal cells in the brain and in the retina.



- Hydramnios T3
- Fetal brain MRI (31 & 35 gw) : white matter anomalies

Normal biometries and gyration
Thin and straight CC

firm and
periventri
atrophic t

mainly found
of neurons

WES: *CTSD* (NM_001909.4)
c.644T>C, p.(Phe215Ser) homozygous

Access to tissues

Human Genetics (2018) 137:175–181
https://doi.org/10.1007/s00439-017-1860-1

ORIGINAL INVESTIGATION

Importance of complete phenotyping in prenatal whole exome sequencing

Mahmoud Aarabi[1,2] · Olivia Sniezek[3,4] · Huaiyang Jiang[4] · Devereux N. Saller[2] · Daniel Bellissimo[1,2] · Svetlana A. Yatsenko[1,2,4,5,6] · Aleksandar Rajkovic[1,2,4,5,6]

47-base pairs deletion in *ZIC2* which was missed by prenatal WES. This study suggests that incomplete prenatal phenotyping and lack of prenatal ultrasound-genotype databases are the limiting factors for current interpretation of WES data in prenatal diagnosis. Development of prenatal phenotype–genotype databases would significantly help WES interpretation in this setting. Patients who underwent prenatal clinical WES may benefit from the re-analysis based on detailed postnatal findings.

Fetal phenotyping

- Prenatal HPO
- Prenatal phenotyping

Welcome back everyone!

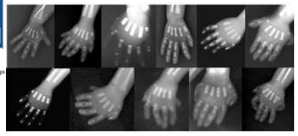
We hope everyone had a good summer. We plan to resume our activities this month with a series of 2-hour workshops workshops will be held, approximately, once a week.

The proposed topics for each meeting are as follows:
1. Refresher, questions (to be held on the week of the 20th of September. Time and date to be announced.)
2. Placental phenotypes
3. Prenatal kidney phenotypes
4. Prenatal skeletal phenotypes
5. Prenatal CNS phenotypes
(If you would like to see another topic here, please let us know.)

Eur J Med Genet. 2012 Feb;55(2):81-90. doi: 10.1016/j.ejmg.2011.12.002. Epub 2011 Dec 22.

Phenotypic spectrum of fetal Smith-Lemli-Opitz syndrome

Chloé Quélin [1], Philippe Loget, Alain Verloes, Anne Bazin, Bettina Bessières, Annie Laquerrière, Sophie Patrier, Romulus Grigorescu, Ferechté Encha-Razavi, Sophie Delahaye, Jean-Marie Jouannic, Bruno Carbonne, Dominique D'Hervé, Marie-Cécile Aubry, Guillaume Mace, Thierry Harvey, Yves Ville, Geraldine Viot, Nicole Joye, Sylvie Odent, Tania Attié-Bitach, Claude Wolf, Françoise Chevy, Pascale Benlian, Marie Gonzales



WES: fetal exome sequencing

- Improves of prenatal management
- Still challenging
- **VUS not returned**
- **Possible subsequent reanalysis**

- Development rapid analytical and interpretation pipelines
- **Better knowledge on prenatal signs of genetic disorders**

- **Following TOP, fetal examination is important**
  - **Accurate phenotyping**
  - **Reverse phenotyping**
  - **Access to tissue**

- **If negative :** further reinterpretation / analysis
- **If positive :** consolidate the genetic result / incomplete phenotypes / atypical signs /
- **contribute to a better knowledge of prenatal signs of rare disorders**