

Le séquençage d'exome dans le bilan des syndromes polymalformatifs

Nicolas BOUCHON - Gaëlle LAMBERT

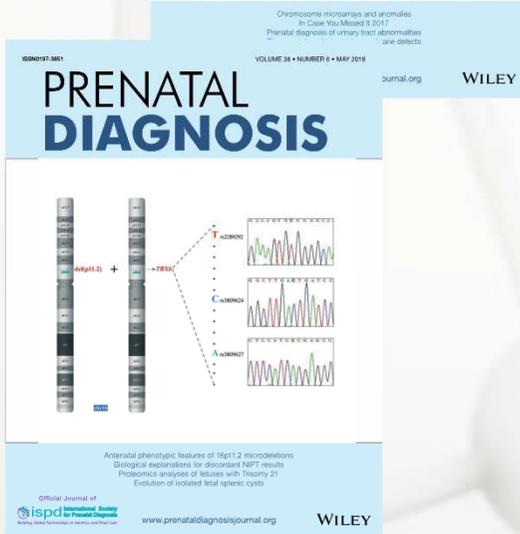
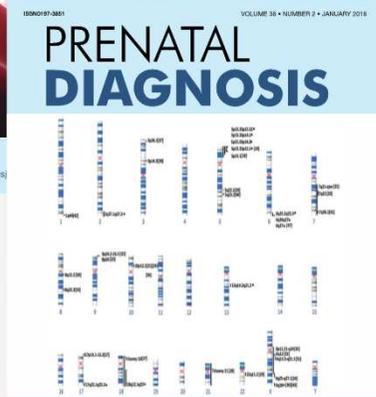
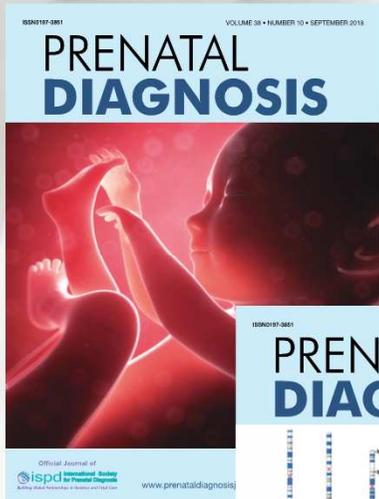
Service de Gynécologie Obstétrique - CHU Dijon / INSERM UMR 1231

Service de Génétique Clinique – CHU Nancy

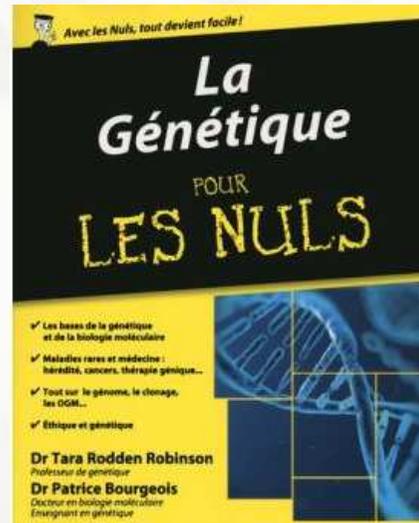
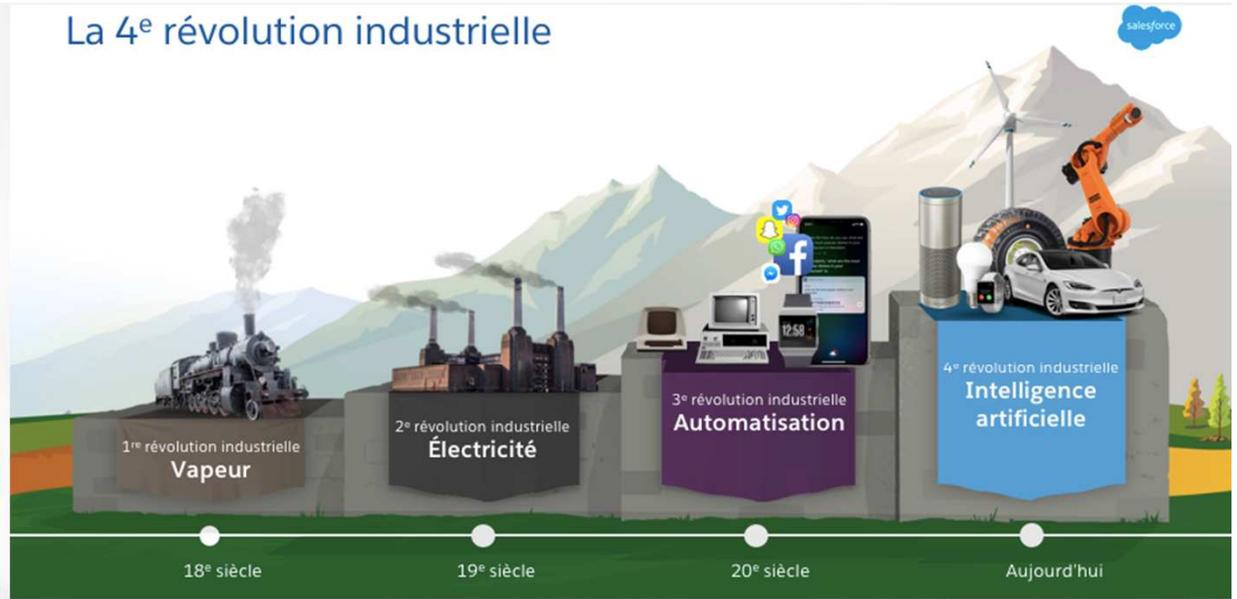
Journée annuelle du CPDP de Strasbourg - Section du Grand Est des CPDP



UNIVERSITÉ
BOURGOGNE-FRANCHE-COMTE



La 4^e révolution industrielle



Aucun conflit d'intérêt

Sommaire

1. Syndromes poly-malformatifs
2. Séquençage nouvelle génération
3. Exome et SPM
4. Perspectives
5. Conclusion

SPM

NGS

WES ET SPM

PERSPECTIVES

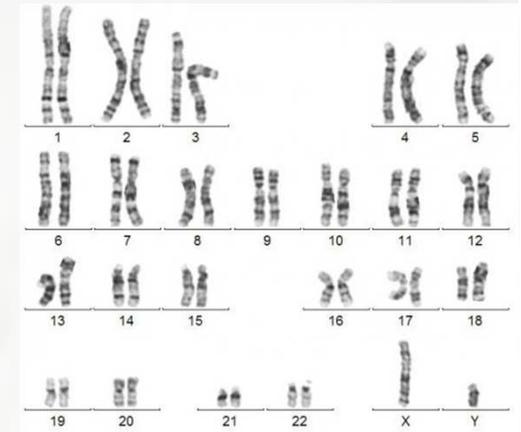
CONCLUSION

Syndromes poly-malformatifs

Au moins deux malformations
congénitales
3 – 5% des naissances
20% de pertes fœtales

Anomalies du développement

Retard de croissance
Dysmorphie
Syndrome poly-malformatif
Déficience intellectuelle



HETEROGENEITE PHENOTYPIQUE HETEROGENEITE GENETIQUE

90% anomalies génétiques

Anomalies chromosomiques
Microremaniements
chromosomiques (CNV)
Anomalies monogéniques
(SNV)

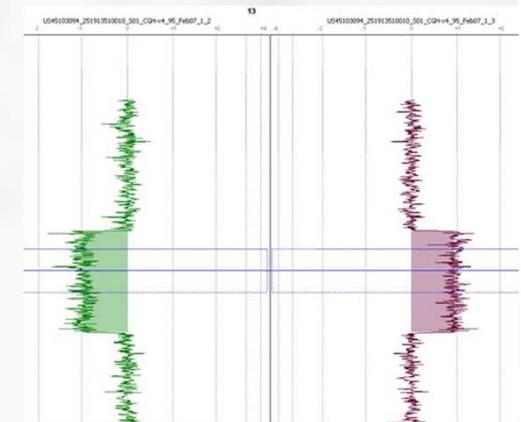
Constitutionnelles
Mosaïque

Techniques

Caryotype standard / FISH

Analyse chromosomique sur
Puces à ADN

Séquençage ADN



TV - 3784_SCN7A_14_MIRROR-1_68.ab1

t View Finch Help



Go to Base No. Find Sequence

Bilan des syndromes poly-malformatifs

ANTENATAL

- Echographie +/- imagerie complémentaire
- Biologie sur liquide amniotique :
 - Génétique : Caryotype/FISH et CGH-array
 - Métabolique, infectieux, biochimique ...

POSTNATAL

- Foetopathologie +++
 - Hypothèse diagnostique : test ciblé
 - Absence d'hypothèse diagnostique : CGH-array +/- panel de gènes

Equipe multidisciplinaire

Consultations pré-test / post-test / synthèse

1- Caryotype

of hands and feet, and growth retardation. In 2086 fetuses with trisomy 21, the associated defects included nuchal oedema, atrioventricular septal defects, mild mental retardation, clinodactyly, and sandal gap. The frequency of chromosomal abnormalities increased with gestational age, but if fetal karyotyping had been

ultrasonographically detectable phenotype. The different types of chromosomal abnormalities were aimed to achieve these two objectives: to identify cytogenetic findings in 2086 fetuses with malformations, growth retardation, or both. The results of ultrasound examination.

- 2086 grossesses avec malformations congénitales
- ≈ 15% d'anomalies chromosomiques :
 - Anomalies de nombre : T13, T18, T21, monosomie X, triploïdie
 - Anomalies de structure : Translocations déséquilibrées, délétions, marqueurs chromosomiques

Prospective ranking of the sonographic markers for aneuploidy: Data of 2143 prenatal cytogenetic diagnoses referred for abnormalities on ultrasound

Art Daniel, Neil Athayde, Robert Ogle, Alice M. George, Jonathan Michael, Mark d Pertile, Jennifer Bryan, Vapinder Jammu, Brian J. Trudinger

- 2143 grossesses avec malformations congénitales
- ≈ 20 % d'anomalies chromosomiques

2- CGH-array

Prenatal Diagnosis 2012, 32, 986–995

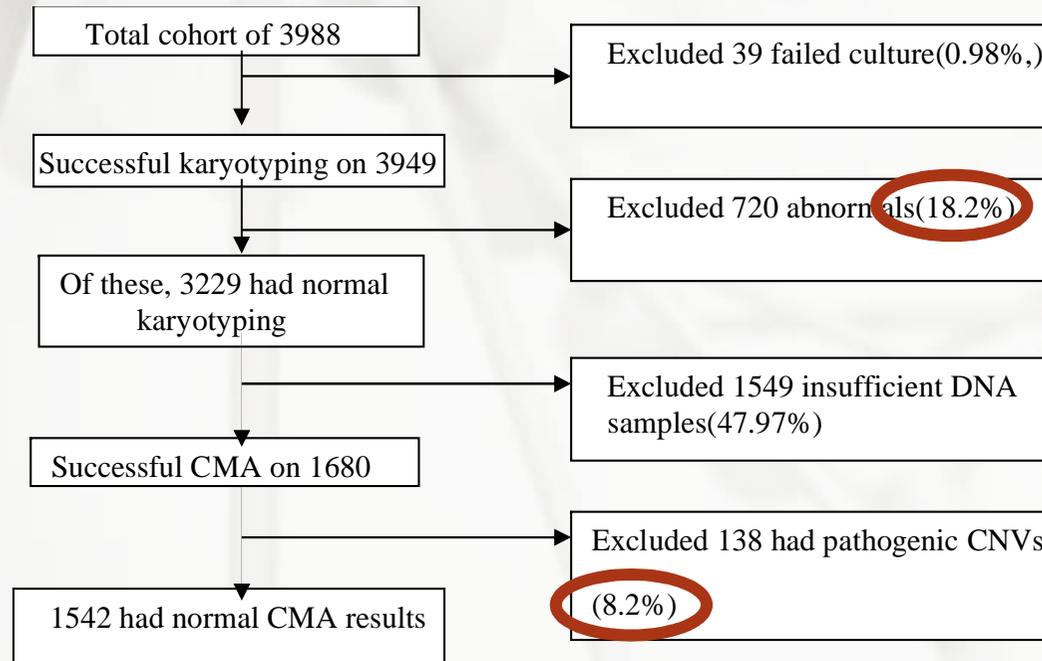
Detection rates of clinically significant genomic alterations by microarray analysis for specific anomalies detected by ultrasound

Lisa G. Shaffer^{1*}, Jill A. Rosenfeld¹, Mindy P. Dabell¹, Justine Coppinger¹, Anne M. Bandholz¹, Jay W. Ellison¹, J. Britt Ravnan¹, Beth S. Torchia¹, Blake C. Ballif¹ and Allan J. Fisher²

- 2858 grossesses avec malformations congénitales à caryotype normal
- Apport de la CGH-array :
 - 6,5 % de diagnostic supplémentaire en MOYENNE
 - Rendement variable

Ultrasound category	Microarray results			TOTAL
	Normal (%)	Unclear (%)	Significant (%)	
Structural abnormalities in multiple systems	492 (85.0)	29 (5.0)	58 (10.0)	579
Structural abnormalities in multiple systems + nonstructural anomalies: TOTAL	196 (85.6)	14 (6.1)	19 (8.3)	229
+ IUGR	17 (77.3)	2 (9.1)	3 (13.6)	22
+ Abnormal amniotic fluid volume	34 (97.1)	0 (0.0)	1 (2.9)	35
+ Soft marker(s)	119 (83.8)	9 (6.3)	14 (9.9)	142
+ Multiple additional findings	26 (86.7)	3 (10.0)	1 (3.3)	30

Quel rendement ?



UOG, 2018

Whole exome sequencing as a diagnostic adjunct to clinical testing in a tertiary referral

cohort of 3988 fetuses with structural abnormalities

Fang Fu^{a1}, Ru Li^{a1}, Yan Li^{b1}, Zhi-Qiang Nie^{c1}, Ting-ying Lei^a, Dan Wang^a, Xin Yang^a, Jin Han^a,

Min Pan^a, Li Zhen^a, Yan-mei Ou^a, Jian Li^a, Fao-Tao Li^a, Xiang-yi Jing^a, Dong-zhi Li^{a#}, Can

Liao^{a#}.

≈ 20 % de diagnostic en cytogénétique conventionnelle

≈ 10 % de diagnostic en cytogénétique moléculaire

30 % de diagnostic

2/3 des couples sans réponse

SPM

NGS

WES ET SPM

PERSPECTIVES

CONCLUSION

Evolution de la génétique

J. G. Mendel
Hérédité

1865

**J. D. Watson
F. Crick**
Double hélice

1965

**Human
Genome
Project**
13 ans
> 3 milliards \$

2003

M. Nirenberg
Code
génétique

1966

**Génome
de James
Watson**
2 mois
2 millions \$

2007

F. Sanger
Technique

1975

**Génome
individuel**
15 jours
3000-5000 \$

2013

J. Rommens
CFTR

1989

**Génome
Individuel**
3 jours
1000 \$

2015

**Génome
Individuel**
1h
100 \$

2025

Technique
Sanger



Next Generation Sequencing (NGS)
= Haut débit = massif en parallèle

Préparation
des librairies

- PCR par émulsion : pyro-séquençage, séquençage par libération de protons
- PCR en pont : séquençage par terminateurs réversibles

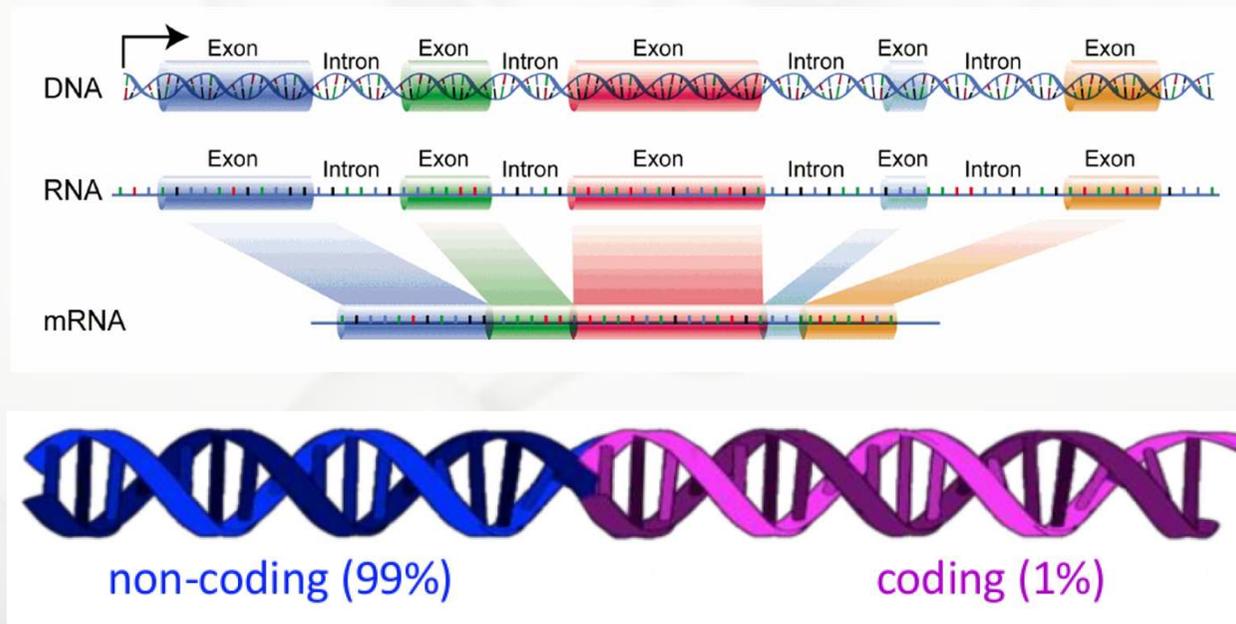
Génération

- 2^{ème} génération : technologies nécessitant un lavage entre chaque cycle
- 3^{ème} génération : technologies ne nécessitant plus de lavage
- 4^{ème} génération : séquençage par libération de protons

Cibles

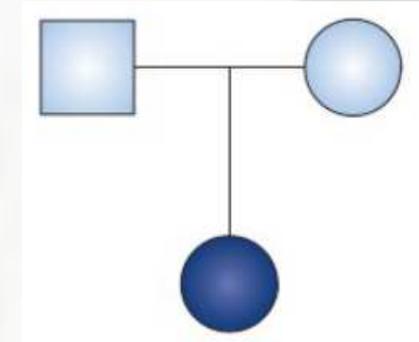
- Génome : Whole Genome Sequencing (WGS)
- Exome : Whole Exome Sequencing (WES)
- ARN : RNA Sequencing (RNA-Seq)...

- Génome humain :
 - $\approx 20\,000$ gènes
 - Gène : séquences codantes ET non codantes
- Exome : ensemble des séquences codantes des gènes



- **2 stratégies principales :**

- Solo : ADN du fœtus
- Trio : ADN du fœtus et des deux parents



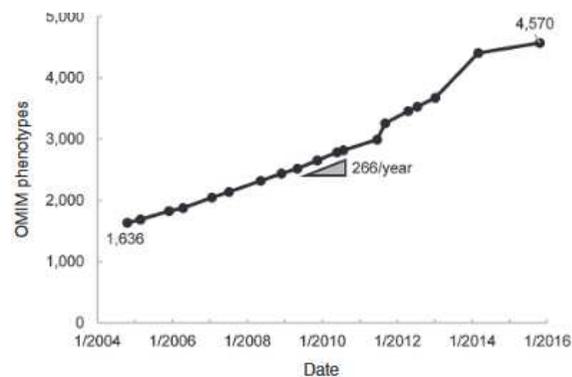
- **Que peut-on détecter avec cette technique ?**

- SNV exoniques
- SNV jonction intron/exon
- CNV exoniques de petite taille (< résolution de la CGH-array)



Contraintes :

- Techniques : qualité/quantité d'ADN, erreurs
- Scientifiques :



Dissected OMIM Morbid Map Scorecard (Updated October 2nd, 2018) :

Class of phenotype	Phenotype	Gene *
Single gene disorders and traits	5,220	3,583
Susceptibility to complex disease or infection	697	503
"Nondiseases"	147	116
Somatic cell genetic disease	211	120

*Some genes may be counted more than once because mutations in a gene may cause more than one phenotype and the phenotypes may be of different classes (e.g., activating somatic BRAF mutation underlying cancer, 164757.0001. and germline BRAF mutation in Noonan syndrome, 164757.0022.)

GAD → **Interprétation des données et corrélation phénotype/génotype**



SPM

NGS

WES ET SPM

PERSPECTIVES

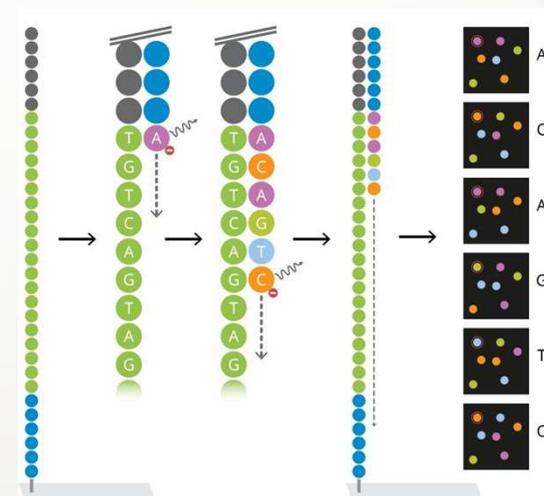
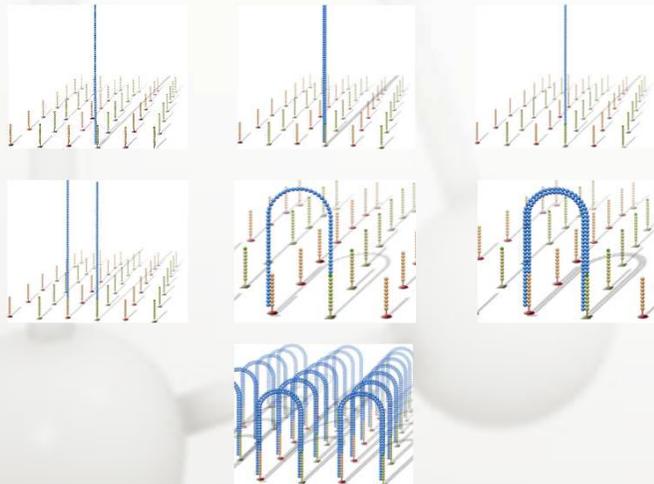
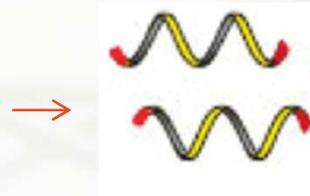
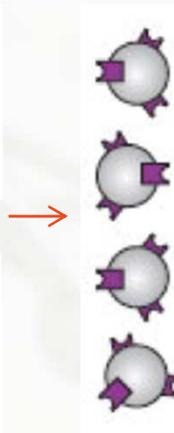
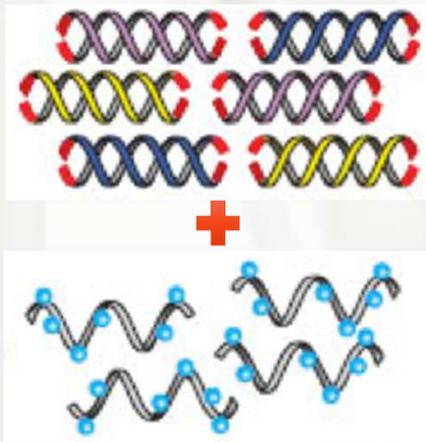
CONCLUSION

Extraction
ADN

Contrôle
qualité

Préparation
des librairies

Séquençage



SPM	NGS	WES ET SPM	PERSPECTIVES	CONCLUSION
-----	------------	------------	--------------	------------



Etapes	Outil
Contrôle qualité des données brutes	FastQC
Elimination des adaptateurs	Trimmomatic
Elimination des bases de mauvaise qualité	Trimmomatic
Alignement par rapport au génome de référence (hg19)	Burrows-Wheeler Aligner
Elimination des duplicats de PCR	Picard
Réalignement, scores de qualité	Genome Analysis ToolKit
Identification SNV	HaplotypeCaller
Identification CNV	eXome-Hidden Markov Model
Annotation SNV	SeattleSeq
Annotation CNV	RefSeq
Annotation complémentaire	Cosmic, ClinVar, OMIM

SPM

NGS

WES ET SPM

PERSPECTIVES

CONCLUSION

Position

Gène,
génotype

Variation, transcrit, protéine, ségrégation

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	
1	#Position	ref/alt	alt	GenomicC	Multiallel	Filter	dijex1602	GeneList	Accession	FunctionGVS	AminoAcid	ProteinPc	cDNAPosi	DistanceT	Truncatin	NSSSI	DeNovo	Recessive
11	chr1:1171427	C/A		chr1:g.11714		PASS	GT=0/1;AD=5	IGSF3	NM_001007237.2,N	missense	ARG/LEU	614/1195,63	1841,1901,19	189	False	True	.	no
25	chr1:2097913	T/C		chr1:g.20979		PASS	GT=0/1;AD=5	LAMB3	NM_000228.2,NM_	(missense	ASP/GLY	982/1173	2945	36	False	True	.	no
29	chr2:2771978	A/ACGGTT		chr2:g.27719		PASS	GT=0/1;AD=1	GCKR	NM_001486.3,XM_	C coding,coding,coding,5	.	.	.	45	False	True	.	no
40	chr2:1109044	G/A		chr2:g.11090		PASS	GT=0/1;AD=7	NPHP1	NM_000272.3,NM_	(intron-near-splice	.	.	.	2	False	True	.	no
42	chr2:1574072	A/G		chr2:g.15740		PASS	GT=0/1;AD=1	GPD2	NM_000408.4,NM_	(missense	ILE/VAL	308/728,308/	922,922,922,;	50	False	True	.	no
44	chr2:1671365	A/G		chr2:g.16713		PASS	GT=0/1;AD=5	SCN9A	NM_002977.3,XM_	C missense	LEU/SER	748/1978,75	2243,2276	68	False	True	.	no
45	chr2:1794281	G/A		chr2:g.17942		PASS	GT=0/1;AD=1	TTN/TTN-A	NM_001256850.1,N	missense,missense,mi	ALA/VAL,AL	25923/34351	77768,82691,	4131	False	True	.	yes
46	chr2:1794602	A/T		chr2:g.17946		PASS	GT=0/1;AD=1	TTN/TTN-A	NM_001256850.1,N	intron-near-splice,intr	.	.	.	4	False	True	.	yes
47	chr2:1794602	C/T		chr2:g.17946		PASS	GT=0/1;AD=1	TTN/TTN-A	NM_001256850.1,N	intron-near-splice,intr	.	.	.	3	False	True	.	yes
51	chr2:2024945	C/T		chr2:g.20249		PASS	GT=0/1;AD=5	TMEM237	NM_001044385.2,N	missense	MET/ILE	206/409,198/	618,594,633	60	False	True	.	no
52	chr2:2192946	T/G		chr2:g.21929		PASS	GT=0/1;AD=5	VIL1	NM_007127.2	intron-near-splice	.	.	.	6	False	True	.	no
54	chr2:2281945	G/GGTGAGC		chr2:g.22819	Multiallelic	PASS	GT=0/1;AD=1	MFF	NM_001277061.1,N	splice-donor,intron,int	.	.	.	0	True	True	.	yes
55	chr2:2282052	T/TACCTGTG		chr2:g.22820		PASS	GT=0/1;AD=5	MFF	NM_001277061.1,N	frameshift-near-splice,	.	.	.	0	True	True	.	yes
82	chr4:7936925	G/A		chr4:g.79369		PASS	GT=0/1;AD=1	FRAS1	NM_025074.6	missense	GLY/ARG	2019/4013	6055	45	False	True	.	no
84	chr4:1046402	C/T		chr4:g.10464		PASS	GT=0/1;AD=4	TACR3	NM_001059.2	intron-near-splice	.	.	.	4	False	True	.	no
95	chr5:7995070	TGCAGCGGC		chr5:g.79950	Multiallelic	PASS	GT=0/2;AD=5	DHFR/MSH	NM_000791.3,NM_	(5-prime-UTR,codingCod	.	.	.	75	False	True	.	no
100	chr5:1372063	C/T		chr5:g.13720		PASS	GT=0/1;AD=7	MYOT	NM_001135940.1,N	intron,missense,intron	none,ARG/C	NA,6/499,NA	NA,16,NA	140	False	True	.	no
103	chr6:1632791	ATGC/ATGCT		chr6:g.16327	Multiallelic	PASS	GT=0/3;AD=4	ATXN1	NM_000332.3,NM_	(codingComplex	.	.	.	786	False	True	.	no
104	chr6:2455694	A/G		chr6:g.24556		PASS	GT=0/1;AD=5	KIAA0319	NM_001168374.1,N	missense	LEU/PRO	906/1064,91	2717,2744,26	10	False	True	.	no
105	chr6:2485282	C/T		chr6:g.24852		PASS	GT=0/1;AD=7	FAM65B	NM_014722.2,NM_	(missense	ARG/HIS	216/1069,21	647,647,734,	19	False	True	.	no
109	chr6:3365585	G/A		chr6:g.33655		PASS	GT=0/1;AD=1	ITPR3	NM_002224.3,XM_	C missense	GLU/LYS	2110/2672,21	6328,6415,35	48	False	True	.	no
112	chr6:8020232	C/T		chr6:g.80202		PASS	GT=0/1;AD=5	LCA5	NM_001122769.2,N	missense	ARG/HIS	301/698	902	44	False	True	.	no
113	chr6:1100363	T/C		chr6:g.11003		PASS	GT=0/1;AD=5	FIG4	NM_014845.5,XM_	C missense,5-prime-UTR	ILE/THR,non	41/908,NA	122,NA	44	False	True	.	no
117	chr6:1192525	G/A		chr6:g.11925		PASS	GT=0/1;AD=5	MCM9	NM_153255.4,XM_	C intron-near-splice	.	.	.	4	False	True	.	no
118	chr6:1319106	T/C		chr6:g.13191		PASS	GT=0/1;AD=7	MED23	NM_001270521.1,N	missense	HIS/ARG	1287/1360,1	3860,3860,38	53	False	True	.	no
119	chr6:1337897	C/T		chr6:g.13378		PASS	GT=0/1;AD=1	EYA4	NM_004100.4,NM_	(missense	THR/MET	289/640,266/	866,797,866,;	62	False	True	.	no
120	chr7:299735	C/T		chr7:g.29973		PASS	GT=0/1;AD=5	FAM20C	NM_020223.3,XM_	C missense	ALA/VAL	515/585,183/	1544,548	39	False	True	.	no
127	chr7:2301803	T/C		chr7:g.23018		PASS	GT=0/1;AD=1	FAM126A	NM_032581.3,XM_	C missense,missense,mi	TYR/CYS,TYR	64/522,64/42	191,191,191,	38	False	True	.	no
133	chr7:9273075	A/G		chr7:g.92730		PASS	GT=0/1;AD=1	SAMD9	NM_001193307.1,N	missense	ILE/THR	1553/1590	4658	4666	False	True	.	no
138	chr7:1288290	G/GGCT		chr7:g.12882		PASS	GT=0/1;AD=5	SMO	NM_005631.4	coding	.	.	.	284	False	True	.	no
142	chr7:1507104	G/A		chr7:g.15071		PASS	GT=0/1;AD=1	NOS3/ATG5	NM_000603.4,NM_	(missense,3-prime-UTR	GLY/GLU,nor	1068/1204,N	3203,NA,272	53	False	True	.	no
145	chr8:1008740	C/T		chr8:g.10087		PASS	GT=0/1;AD=5	VPS13B	NM_017890.4,NM_	(missense	ARG/TRP	3725/4023,3	11173,11098,	54	False	True	.	no
147	chr8:1356148	G/A		chr8:g.13561		PASS	GT=0/1;AD=1	ZFAT	NM_001029939.3,N	missense	ALA/VAL	364/1232,36	1091,1091,94	342	False	True	.	no
151	chr9:2717744	TCAAA/T		chr9:g.27177		PASS	GT=0/1;AD=5	KCNV2	NM_133497.3	frameshift	.	.	.	1348	True	True	.	no
155	chr9:1178046	T/C		chr9:g.11780		PASS	GT=0/1;AD=4	TNC	NM_002160.3,XM_	C missense	MET/VAL	1710/2202,1	5128,4855,40	3	False	True	.	no
156	chr9:1318605	A/G		chr9:g.13186		PASS	GT=0/1;AD=4	CRAT	NM_000755.3,NM_	(missense	PHE/LEU	432/627,411/	1294,1231,12	35	False	True	.	yes
157	chr9:1318625	C/T		chr9:g.13186		PASS	GT=0/1;AD=5	CRAT	NM_000755.3,NM_	(missense	ARG/HIS	277/627,256/	830,767,833,;	25	False	True	.	yes
161	chr9:1401276	G/A		chr9:g.14012		PASS	GT=0/1;AD=5	SLC34A3	NM_001177316.1,N	intron-near-splice	.	.	.	6	False	True	.	no
162	chr10:606630	G/A		chr10:g.6066		PASS	GT=0/1;AD=1	IL2RA	NM_000417.2,XM_	C missense	THR/MET	91/273,91/2	272	16	False	True	.	no

SPM

NGS

WES ET SPM

PERSPECTIVES

CONCLUSION

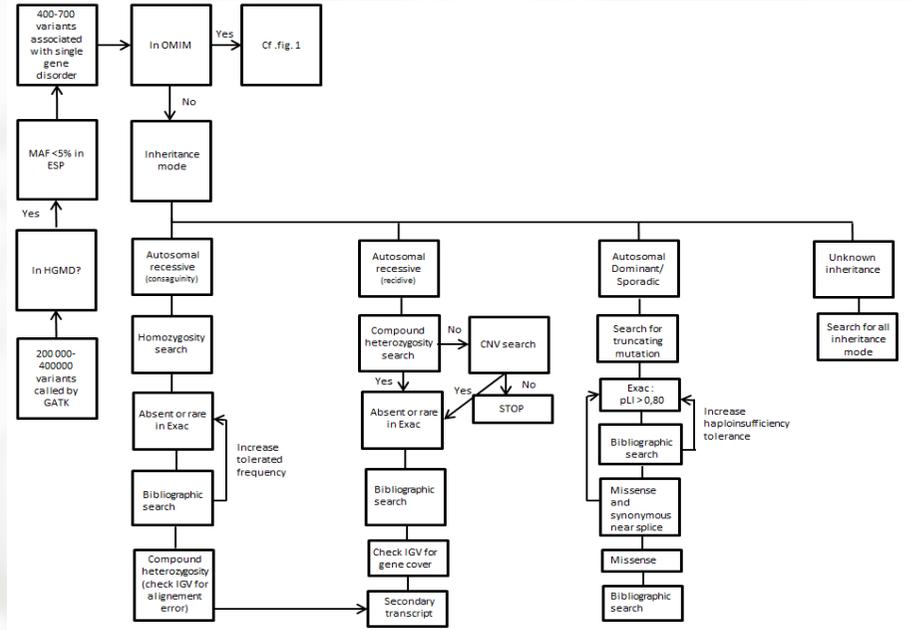
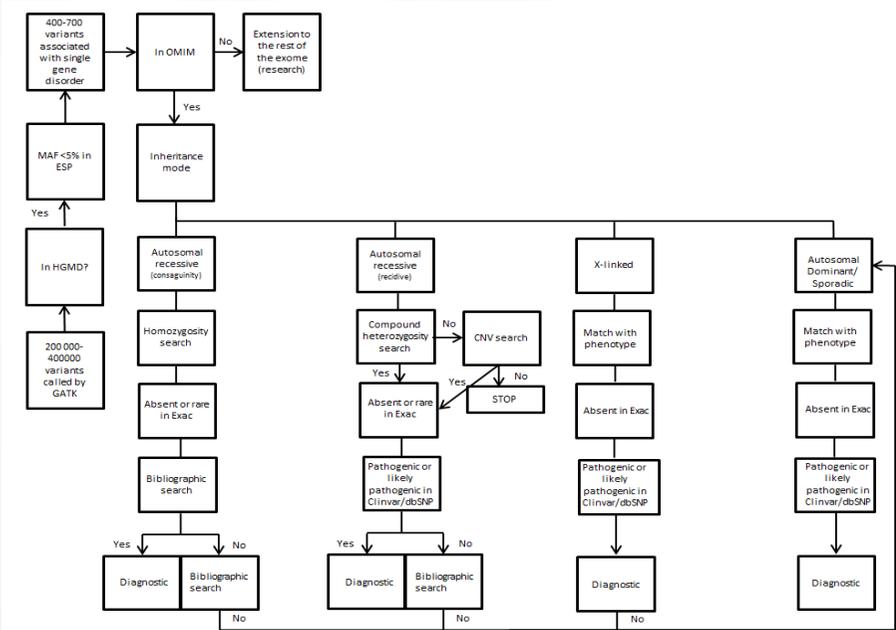
Fréquence

Bases de données

In silico

dbSNP_ID	inDbSNP	allelesDB	genomes	genomes	Freq_GES	Freq_GEX	BatchSam	ControlSa	OMIM	ACMG	ClinVar	COSMIC	scoreCAD	polyPhen	consScor
rs190300523	135	C/A	C:13006	T:2/C:121792	0.0000	0.00000	2	2	?Lacrimal_duct_defect_149700_(3)	.	.	.	27.900	0.997	4.570
rs140769823	134	T/C	C:23/T:12983	C:148/T:12270	0.0018	0.00120	1	1	Amelogenesis_imperfecta_type_1A_104530_(3)	.	.	.	10.230	.	0 4.470
rs748731892	.	.	.	insCGGTTT:2	0.0000	0.00019	1	1	[Fasting_plasma_glucose_level_QTL_5]_613463_	1.310
rs151204566	134	G/A	A:34/G:1297	A:259/G:122	0.0026	0.00211	1	0	Joubert_syndrome_4_609583_(3) Nephronopht	.	Uncertain_si	.	10.720	.	3.070
rs116503732	132	A/G	G:52/A:1295	G:359/A:122	0.0040	0.00293	1	1	{Diabetes_type_2_susceptibility_to}_125853_(.	.	.	21.900	0.107	5.780
rs55634791	129	G/A	A:11838	.	0.0000	0.00000	1	0	Epilepsy_generalized_with_febrile_seizures_pl	.	.	.	21.800	0.995	5.900
rs767769973	.	.	A:5/G:12301	A:90/G:1218	0.0004	0.00074	1	0	Cardiomyopathy_dilated_1G_604145_(3) Cardi	.	Uncertain_si	.	14.220	0.952	5.750
rs761342850	.	.	A:11602	.	0.0000	0.00000	4	0	Cardiomyopathy_dilated_1G_604145_(3) Cardi	.	.	.	8.455	.	3.730
rs769691094	.	.	C:11608	.	0.0000	0.00000	3	0	Cardiomyopathy_dilated_1G_604145_(3) Cardi	.	.	.	10.920	.	5.200
rs746093981	.	.	C:12092	T:1/C:122279	0.0000	0.00001	1	0	Joubert_syndrome_14_614424_(3)	.	.	.	17.670	0.03	5.770
rs770462221	130	del27	T:13006	.	0.0000	0.00000	5	0	Cholestasis_progressive_canalicular_1	.	.	.	0.361	.	-6.780
rs797045409	136	CTG(36_38)	.	.	0.0000:0.0000	0.00000:0.0000	01:00:00	00:01:01	Encephalopathy_due_to_defective_mitochondri	5.730
rs758236733	.	.	G:12136	.	0.0000	0.00000	1	0	Encephalopathy_due_to_defective_mitochondri	5.950
rs763520343	.	.	C:13006	T:1/C:122131	0.0000	0.00001	1	0	Fraser_syndrome_219000_(3)	.	.	.	21.900	.	1 5.680
rs139142572	134	C/T	T:5/C:12971	A:1/T:46/C:5	0.0004	0.00089	1	0	Hypogonadotropic_hypogonadism_11_with_or_v	.	.	.	12.530	.	-1.540
rs121908287	133	T/C	C:13006	delGCAGCGC	0.0000:0.0000	0.01021:0.00	02:01:00	02:00:02	Megaloblastic_anemia_due_to_dihydrofolate_re	.	.	COSM16665	.	.	.
rs150598354	134	T/C	A:13006	.	0.0000	0.00000	1	0	Muscular_dystrophy_limb-girdle_type_1A_159	.	.	.	22.100	.	1 5.490
rs41286200	127	C/T	.	.	0.0000:0.0000	0.00000:0.00	4:1:0:1:0	22:13:2:2:1:2	Spinocerebellar_ataxia_1_164400_(3)	.	Uncertain_si	COSM371	.	.	0.046
rs146913158	134	T/C	C:12032	A:1/T:1/C:12	0.0000	0.00001	1	0	{Dyslexia_susceptibility_to}_2_600202_(3)	.	.	.	14.840	.	1 4.020
rs146732631	134	A/G	G:13006	A:6/G:12254	0.0000	0.00005	1	0	?Deafness_autosomal_recessive_104_616515_(.	.	.	32.000	.	1 5.570
rs570242755	.	.	G:13006	A:6/G:12254	0.0000	0.00005	1	0	{Diabetes_type_1_susceptibility_to}_222100_(.	.	.	8.932	0.009	4.850
rs779427167	137	G/A	T:5/C:12971	A:1/T:46/C:5	0.0004	0.00089	1	0	Leber_congenital_amaurosis_5_604537_(3)	.	.	.	23.100	.	1 5.390
rs199724546	137	G/A	C:15/T:12989	C:119/T:1215	0.0012	0.00098	1	0	?Polymicrogyria_bilateral_temporooccipital_61	.	Pathogenic	.	22.800	.	1 5.680
rs780555806	.	.	G:13000	.	0.0000	0.00000	2	0	Ovarian_dysgenesis_4_616185_(3)	.	.	.	5.610	.	2.250
rs201311843	137	T/C	C:9/T:12997	C:54/T:12250	0.0007	0.00044	1	0	Mental_retardation_autosomal_recessive_18_6	.	Uncertain_si	.	13.070	0.612	5.230
rs140551503	134	A/G	T:8/C:12996	T:78/C:12258	0.0006	0.00064	1	1	Cardiomyopathy_dilated_1J_605362_(3) Deafn	.	Uncertain_si	.	22.400	0.997	5.730
rs146338921	134	C/T	C:4566	.	0.0000	0.00000	1	0	Raine_syndrome_259775_(3)	.	.	.	17.090	.	1 4.520
rs756580408	134	T/C	C:16/T:12990	C:165/T:1215	0.0012	0.00136	1	0	Leukodystrophy_hypomyelinating_5_610532_(.	.	.	21.300	.	1 5.860
rs72650666	130	G/A	G:1/A:13005	G:11/A:1228	0.0001	0.00009	1	0	MIRAGE_syndrome_617053_(3) Tumoral_calcinc	.	.	.	11.630	0.816	4.340
rs199724546	137	G/A	G:13006	.	0.0000	0.00000	2	2	Basal_cell_carcinoma_somatic_605462_(3) Curr	.	.	COSM11696	.	.	3.740
rs780555806	.	.	C:12996	T:5/C:52605	0.0000	0.00010	1	0	{Alzheimer_disease_late-onset_susceptibility	.	.	.	20.900	0.97	4.180
rs201311843	137	T/C	C:12996	T:5/C:52605	0.0000	0.00010	1	0	Cohen_syndrome_216550_(3)	.	.	.	24.700	.	1 4.860
rs140551503	134	A/G	A:3/G:12699	A:9/G:12220	0.0002	0.00007	1	0	{Autoimmune_thyroid_disease_susceptibility_t	.	.	COSM54821	6.017	0.816	3.940
rs146338921	134	C/T	.	.	0.0000	0.00000	1	0	Retinal_cone_dystrophy_3B_610356_(3)	.	Pathogenic	.	.	.	4.920
rs756580408	.	.	C:4/T:13002	C:42/T:12240	0.0003	0.00034	1	0	Deafness_autosomal_dominant_56_615629_(3)	.	.	.	2.950	0.007	4.960
rs72650666	130	G/A	G:5/A:13001	G:56/A:1226	0.0004	0.00046	1	0	?Carnitine_acetyltransferase_deficiency_1	.	.	.	28.700	0.991	5.130
rs199724546	137	G/A	T:6/C:13000	T:62/C:11908	0.0005	0.00052	1	0	?Carnitine_acetyltransferase_deficiency_1	.	.	.	14.050	0.003	2.690
rs780555806	.	.	G:12048	A:2/G:24054	0.0000	0.00008	1	0	Hypophosphatemic_rickets_with_hypercalciuria	.	.	.	8.137	.	2.430
rs201311843	137	T/C	A:11/G:1299	A:212/G:122	0.0008	0.00173	1	0	Immunodeficiency_41_with_lymphoproliferatio	.	.	.	7.324	0.997	-5.470

SPM	NGS	WES ET SPM	PERSPECTIVES	CONCLUSION
-----	-----	------------	--------------	------------



Pourquoi l'exome et non pas le génome ?

- 85% des mutations rapportées altèrent le cadre de lecture
- On ne peut pas encore faire du génome en routine
- Technique performante :
 - Amélioration du rendement diagnostique
 - Identification de nouveaux gènes

The oral-facial-digital syndrome gene *C2CD3* encodes a positive regulator of centriole elongation

Christel Chauvin-Robinet^{1,2,17}, Jaclyn S Lee^{3,17}, Estelle Lopez¹, Vicente Herranz-Pérez^{4,5}, Toshinobu Shida³, Brunella Franco^{6,7}, Laurence Jegou¹, Fan Ye³, Laurent Pasquier⁸, Philippe Loget⁹, Nadège Gigot^{1,10}, Bernard Aral^{1,10}, Carla A M Lopes¹¹, Judith St-Onge^{1,10}, Ange-Line Bruel¹, Julien Thevenon^{1,2}, Susana González-Granero^{4,5}, Caroline Alby^{12,13}, Arnold Munnich¹²⁻¹⁴, Michel Vekemans¹²⁻¹⁴, Frédéric Huet^{1,2}, Andrew M Fry¹¹, Sophie Saunier^{13,15}, Jean-Baptiste Rivière^{1,10}, Tania Attié-Bitach¹²⁻¹⁴, Jose Manuel Garcia-Verdugo^{4,5}, Laurence Faivre^{1,2}, André Mégarbané¹⁶ & Maxence V Nachury³

The contribution of *de novo* coding mutations to autism spectrum disorder

Ivan Iossifov^{1*}, Brian J. O'Roak^{2-3*}, Stephan J. Sanders^{4,5*}, Michael Ronemus^{1*}, Niklas Krumm², Dan Levy¹, Holly A. Stessman², Kali T. Witherspoon¹, Laura Vives¹, Karynne E. Patterson², Joshua D. Smith¹, Bryan Paeppe², Deborah A. Nickerson², Jeanselle Dea¹, Shan Dong²⁻⁶, Luis E. Gonzalez⁷, Jeffrey D. Mandel⁸, Shrikant M. Nane⁹, Michael T. Murtha¹, Catherine A. Sullivan¹, Michael F. Walker¹, Zaimulabedin Waqar¹, Liping Wei^{5,9}, A. Jeremy Willsey^{4,5}, Boris Yamrom¹, Yoon-ha Lee¹, Ewa Grabowska^{1,10}, Ertugrul Dalkic^{1,11}, Zihua Wang¹, Steven Marks¹, Peter Andrews¹, Anthony Leotta¹, Jude Kendall¹, Inessa Hakker¹, Julie Rosenbaum¹, Beicong Ma¹, Linda Rodgers¹, Jennifer Troge¹, Giuseppe Narzisi^{1,10}, Seungtae Yoon¹, Michael C. Schatz¹, Kenny Ye^{1,2}, W. Richard McCombie¹, Jay Shendure², Evan E. Eichler^{2,13}, Matthew W. State^{4,5,7,14} & Michael Wigler¹

Targeted Next-Generation Sequencing Identifies Pathogenic Variants in Familial Congenital Heart Disease

Gillian M. Blue, MSc,[†] Edwin P. Kirk, MBBS, PhD,[‡] Eleni Giannoulatou, MEng, MPhil, DPhil,^{‡*} Sally L. Dunwoodie, PhD,^{‡††} Joshua W.K. Ho, PhD,^{‡*} Desiree C.K. Hilton, PhD,[†] Susan M. White, MBBS Gary F. Sholler, MBBS (Hons),[†] Richard P. Harvey, PhD,^{‡††} David S. Winlaw, MBBS, MD[†]

Genetic studies in intellectual disability and related disorders

Lisenka E. L. M. Vissers¹, Christian Gilissen¹ and Joris A. Veltman^{1,2}

Exome sequencing identifies *MLL2* mutations as a cause of Kabuki syndrome

Sarah B Ng^{1,7}, Abigail W Bigham^{2,7}, Kati JBuckingham², Mark CHannibal^{2,3}, Margaret JMcMillin², Heidi I Gildersleeve², Anita E Beck^{2,3}, Holly K Tabor^{2,3}, Gregory M Cooper¹, Heather C Mefford², Choli Lee¹, Emily H Turner¹, Joshua D Smith¹, Mark J Rieder¹, Koh-ichiro Yoshiura⁴, Naomichi Matsumoto⁵, Tohru Ohta⁶, Norio Niikawa⁴, Deborah A Nickerson¹, Michael JBamshad¹⁻³ & Jay Shendure¹

Diagnostic exome sequencing provides a molecular diagnosis for a significant proportion of patients with epilepsy

Katherine L. Helbig, MS¹, Kelly D. Farwell Hagman, MS¹, Deepali N. Shinde, PhD¹, Cameron Mroske, MSc¹, Zöe Powis, MS¹, Shuwei Li, PhD², Sha Tang, PhD¹ and Ingo Helbig, MD^{3,4}



WES et syndromes poly-malformatifs

PAGE

Prenatal Assessment of
Genomes and Exomes

[Home](#)[About](#)[For Parents](#)[For Researchers](#)

Striving for a better understanding of genetic variants causing developmental problems during pregnancy.

Aiming to improve prenatal diagnostics and parental counselling in the future.

**OBJECTIF : 1000 familles sur signes
d'appel échographique
Débuté en 2014, pas encore de résultats
définitifs**



The 100,000 Genomes Project

Genomics England & Partners



SPM	NGS	WES ET SPM	PERSPECTIVES	CONCLUSION
-----	-----	-------------------	--------------	------------

Que dit la littérature ?

Reference	Nombre de cas	Stratégie ES	Variation pathogène	Variation probablement pathogène
Yang et coll., 2014	11	Trio	6/11 (54%)	-
Carss et coll., 2014	30	Trio	3/30 (10%)	5/30 (16.7%)
Drury et coll., 2015	24	14 solo 10 trio	5/24 (20.8%)	1/24 (4.2%)
Alamillo et coll., 2015	7	Trio	3/7 (42.9%)	1/7 (14.3%)
Drury et coll., 2016	6	Trio	3/6 (50%)	-
Wapner et coll. 2017	168	Trio	13/168 (7.7%)	30/168 (17.9%)
Pangalos et coll., 2016	14	Solo	6/14 (42.9%)	-
Yates et coll., 2017	84	33 solo/duo 51 trio/quattor	17/84 (20%)	38/84 (45%)
Vora et al., 2017	15	Trio	7/15 (46.7%)	1/15 (6.7%)
Fu et coll., 2018	196	34 solo 13 trio	47/196 (24%)	25/196 (12.8%)
Daum et coll., 2018	77	14 solo 63 trio	Consanguin 7/14 (50%) Solo 11/44 (25%) Trio 5/34 (15%)	-
Arabi et coll., 2018	20	Trio	1/20 (5%)	2/10 (10%)

Rendement : 25 % - 35 % selon la stratégie et le phénotype sélectionné



SPM

NGS

WES ET SPM

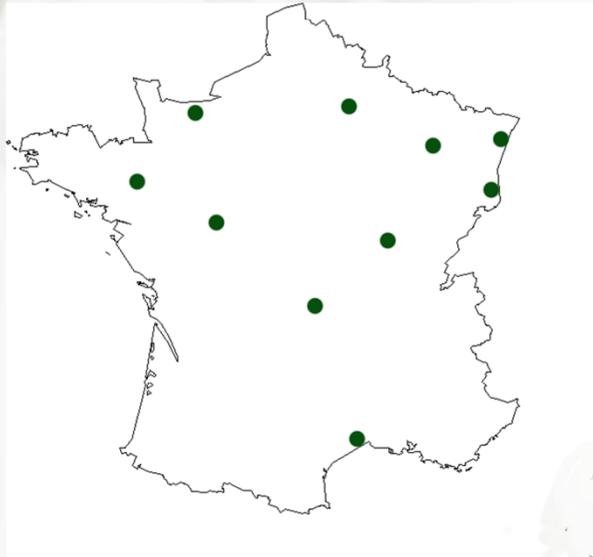
PERSPECTIVES

CONCLUSION

PHRC national « FOETEX »

Investigateurs principaux :

Dr Julien THEVENON et Dr Mathilde LEFEBVRE

**PHRC FOETEX**

Critères d'inclusion

- Syndrome poly-malformatif
- Absence d'hypothèse diagnostique
- Caryotype standard normal
- ACPA normale

Critères d'exclusion

- Refus des parents
- Hypothèse diagnostique : séquençage ciblé

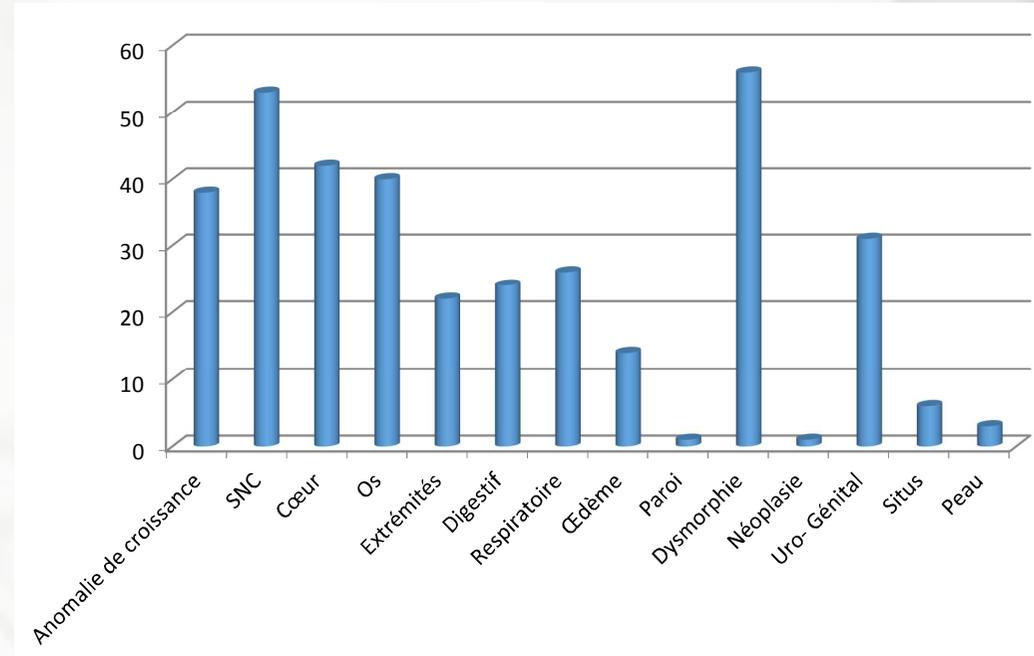
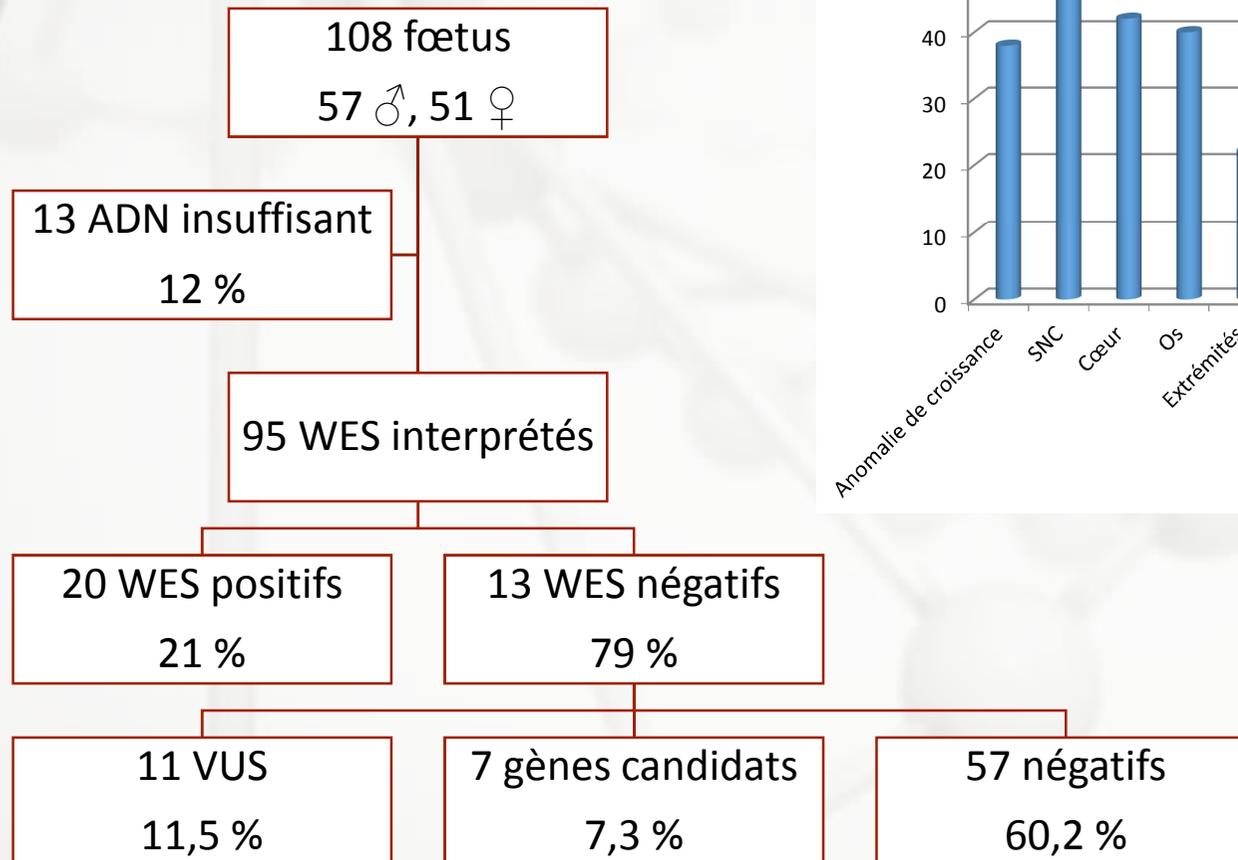
DIAGNOSTIC

- Identifier un variant dans un gène connu en pathologie humaine compatible avec le phénotype clinique

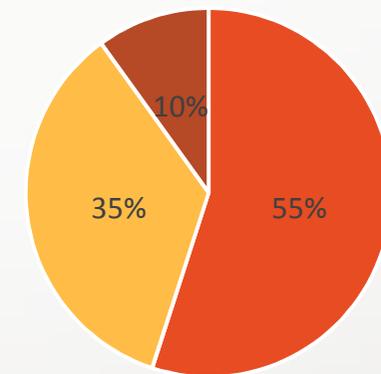
RECHERCHE

- Identifier le rôle de gènes non encore connus en pathologie humaine
- Elargir le spectre phénotypique des gènes connus en pathologie humaine

Fœtopathologie



Transmission



■ AR ■ Sporadique ■ AD

SPM

NGS

WES ET SPM

PERSPECTIVES

CONCLUSION

Phénotypes typiques



FGFR2: p.Ser351Cys
Pfeiffer type III Syndrome

RCIU, Craniosténose

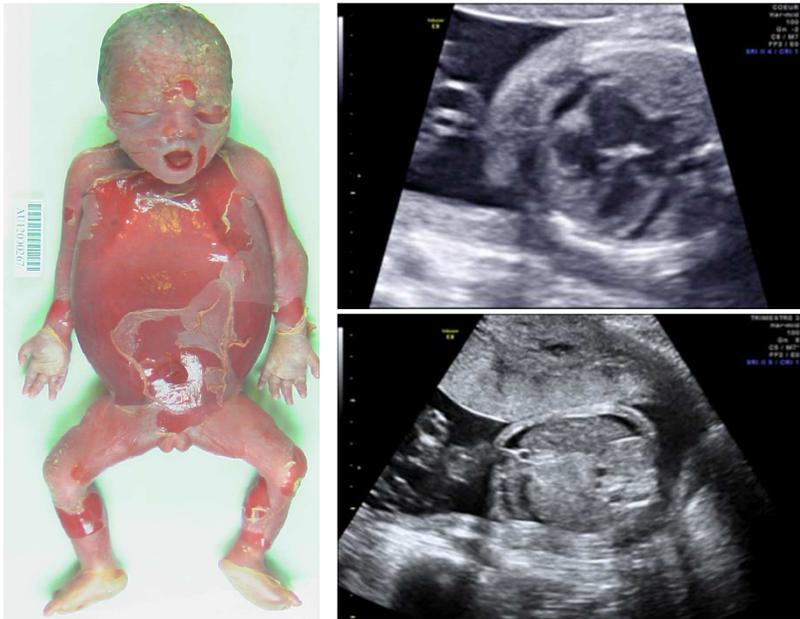
Phénotypes aspécifiques



B3GALT1: c.660+1G>A/p.Arg375*
Peters Plus Syndrome

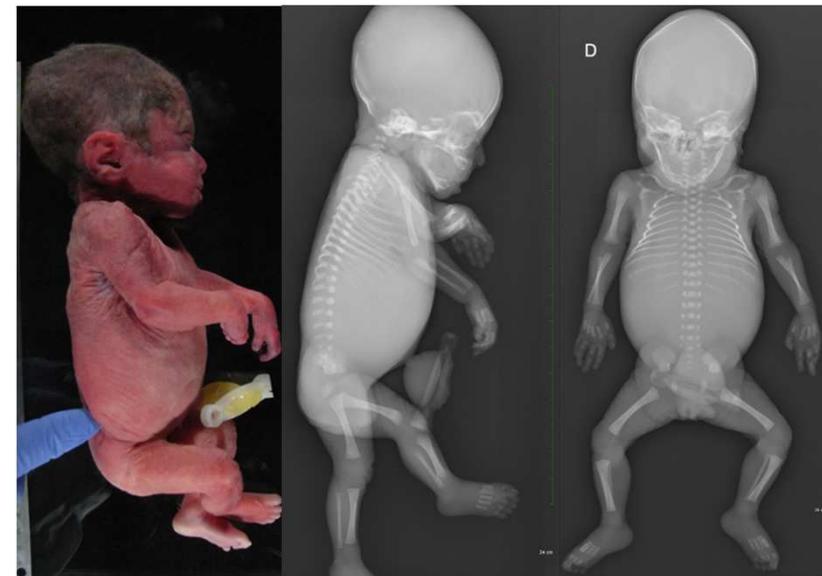
RCIU, fente labio-palatine, hypoplasie du
CC

Phénotypes extrêmes ou atypiques



TREX1 : p.Leu123Cysfr*27
Syndrome d'Aicardi Goutière

RCIU, microcéphalie, calcification NGC



ALDH18A1 : p.(Arg425Cys) et p.(Lys59Asnfs*9)
Cutis Laxa IIIA

RCIU, ACC, dysmorphie, hydronéphrose, os
longs courts, côtes courtes

Simulation DPN

MATERIEL ET METHODES

Cas positifs
Données prénatales

Double lecture en aveugle
2 binômes de 2 lecteurs
Rendu des résultats en RCP

- 15 dossiers complets
- Concordance :
 - 13/15 entre les deux lecteurs
 - 15/15 lors de la RCP clinico-biologique

Perspectives pour les cas négatifs

Rendement diagnostique = 21%

1

- Ré-analyse individuelle
- Ré-analyse globale

2

- Recherche de mosaïque

3

- WES en trio

4

- WGS et RNA-Seq

Clinical whole-exome sequencing for the diagnosis of rare disorders with congenital anomalies and/or intellectual disability: substantial interest of prospective annual reanalysis

Sophie Nambot, MD^{1,2,3,4}, Julien Thevenon, MD, PhD^{1,3,4}, Paul Kuentz, MD, PhD^{2,3,4}, Yannis Duffourd, MSc^{3,4}, Emilie Tisserant, PhD^{3,4}, Ange-Line Bruel, PhD^{3,4}, Anne-Laure Mosca-Boidron, MD^{2,3,4}, Alice Masurel-Paulet, MD^{1,3}, Daphné Lehalle, MD¹, Nolwenn Jean-Marçais, MD^{1,3}, Mathilde Lefebvre, MD^{1,2}, Pierre Vabres, MD, PhD^{3,4}, Salima El Chehadeh-Djebbar, MD¹, Christophe Philippe, MD, PhD^{2,4}, Frederic Tran Mau-Them, MD^{2,4}, Judith St-Onge, DEC^{2,4}, Thibaud Jouan, BSc^{2,3,4}, Martin Chevarin, HNC^{2,3,4}, Charlotte Poé, BSc^{2,3,4}, Virginie Carmignac, PhD⁴, Antonio Vitobello, PhD^{2,3,4}, Patrick Callier, MD, PhD^{2,3,4}, Jean-Baptiste Rivière, PhD^{2,3,4}, Laurence Faivre, MD, PhD^{1,3,4}, Christel Thauvin-Robinet, MD, PhD^{1,2,3,4} and Orphanomix Physicians' Group⁸

Whole-genome sequencing offers additional but limited clinical utility compared with reanalysis of whole-exome sequencing

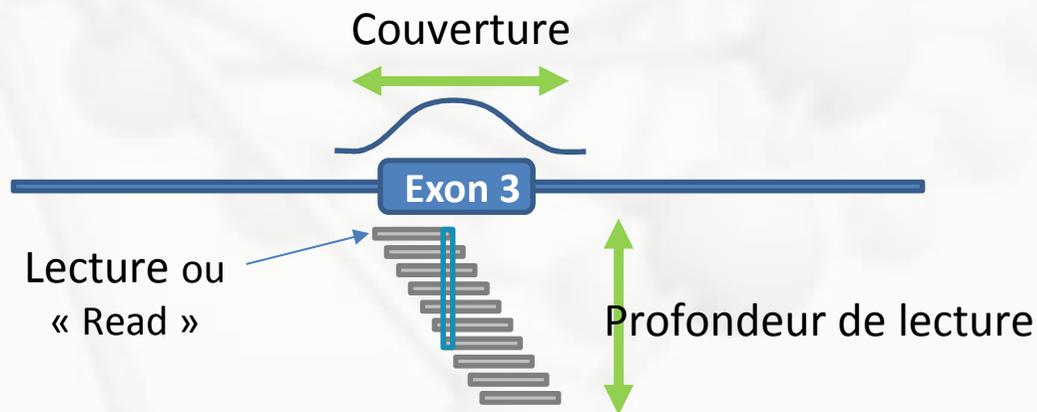
Ahmed Alfares, MD, FACMG¹, Taghrid Aloraini, MS², Lamia Al subaia, MS³, Abdulelah Alissa, MS², Ahmed Al Qudsi, MS², Ahmed Alahmad, MS², Fuad Al Mutairi, MD^{3,4}, Abdulrahman Alswaid, MD^{3,4}, Ali Alothaim, MD^{2,4}, Wafaa Eyaad, MD^{3,4}, Mohammed Albalwi, PhD^{2,4}, Saeed Alturki, MD, PhD^{2,4} and Majid Alfadhel, MD^{3,4}

Whole-genome sequencing is more powerful than whole-exome sequencing for detecting exome variants

Aziz Belkadi^{a,b,1}, Alexandre Bolze^{c,1,2}, Yuval Itan^c, Aurélie Cobat^{a,b}, Quentin B. Vincent^{a,b}, Alexander Antipenko^c, Lei Shang^f, Bertrand Boisson^f, Jean-Laurent Casanova^{a,b,c,d,e,3,4}, and Laurent Abel^{a,b,c,3,4}

Avantages du WGS / WES

- Meilleure couverture de l'exome
---> 3 % de SNV exoniques en plus



- Analyse des régions introniques : 15% des SNV pathogènes
- Recherche anomalies de structure

SPM

NGS

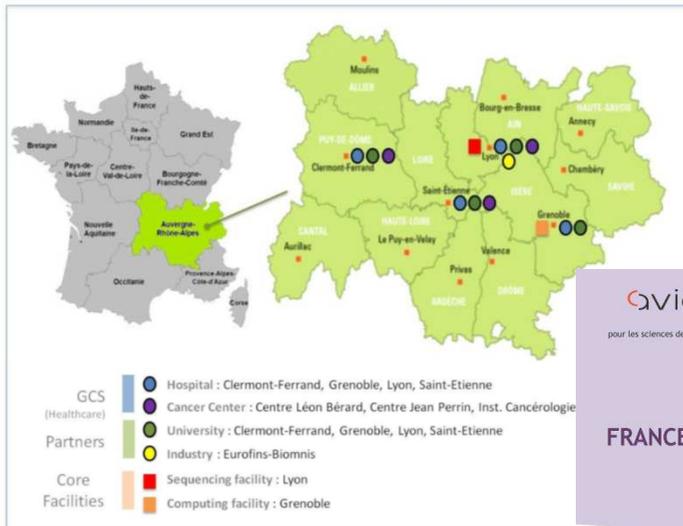
WES ET SPM

PERSPECTIVES

CONCLUSION

Plan FMG

PLATEFORME AURAGEN



PROJET SEQOIA - IDF

ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS



GUSTAVE ROUSSY

CANCER CAMPUS GRAND PARIS

institutCurie



SPM

NGS

WES ET SPM

PERSPECTIVES

CONCLUSION

Projet de recherche



AnDDI-Rares

Investigateurs principaux :

Pr Christel THAUVIN – Dr Nicolas BOURGON

PHRC ANDDI-PRENATOME

Critères d'inclusion

- Au moins 2 malformations
- Indication de prélèvement invasif

Critères d'exclusion

- Refus des parents
- Hypothèse diagnostique : séquençage ciblé

OBJECTIF PRINCIPAL

Evaluer de façon pilote la capacité à rendre un WGS rapide en situation de diagnostic prénatal

Inclusion : 50 fœtus

Comparer 2 stratégies :

- WGS en trio sur LA
- WGS en trio sur ADN foetal circulant

Syndromes poly-malformatifs

- Hétérogénéité clinique et génétique/moléculaire
- Nécessité d'améliorer le conseil génétique pour 2/3 des couples

Séquençage d'exome

- Première étape de l'application du NGS
- Approche trio semble être supérieure au solo
- Faisabilité / coûts

Malgré ça

- 50% de parents sans réponse
- Stratégie complémentaire : ré analyse / WGS ?
- NGS ne résout pas tout : anomalies cytogénétiques

Merci de votre attention

Laurence Faivre

Christel Thauvin-Robinet

Christophe Philippe

Frédéric Huet

Patricia Fauque

Pierre Vabres

Patrick Caillier

Paul Kuentz

Céline Bruno

Cécile Choux

Frédéric Tran-Mau Them

Arthur Sorlin

Nicolas Bourgon

Sophie Nambot

Mathilde Lefebvre

Yannis Duffourd

Emilie Tisserant

Virginie Quéré

Ronan Quéré

Laurence Jego

Ange-Line Bruel

Antonio Vitobello

Romain Da Costa

Philippine Garret

Vincent Lhussiez

Alexandre Plagioz

Sébastien Moutton

Thibaud Jouan

Charlotte Poë

Martin Chevarin

Morgane Bordessoules

Justine Lavoyer

