Prenatal array

The Belgian consensus

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Change of paradigm's in obstetric genetics in the past

- **Invasive techniques**

– amniocentesis (70s)

– fetoscopy (70s)

– skinbiopsy (80s)

– CVS (80s)

–Percutaneous umbilical cord sampling (PUBS) (80-90s)

- **Non-invasive techniques**

– maternel serum AFP for NTD (80s)

– ultrasound (80s)

– Triple/Quad screen for DS (80-90s)

– First trimester screening for DS and trisomy 13/18 (2000)

– NIPT (2011)

- **Preimplantation genetic diagnosis (90s)**

Recent changes of paradigm's in obstetric genetics

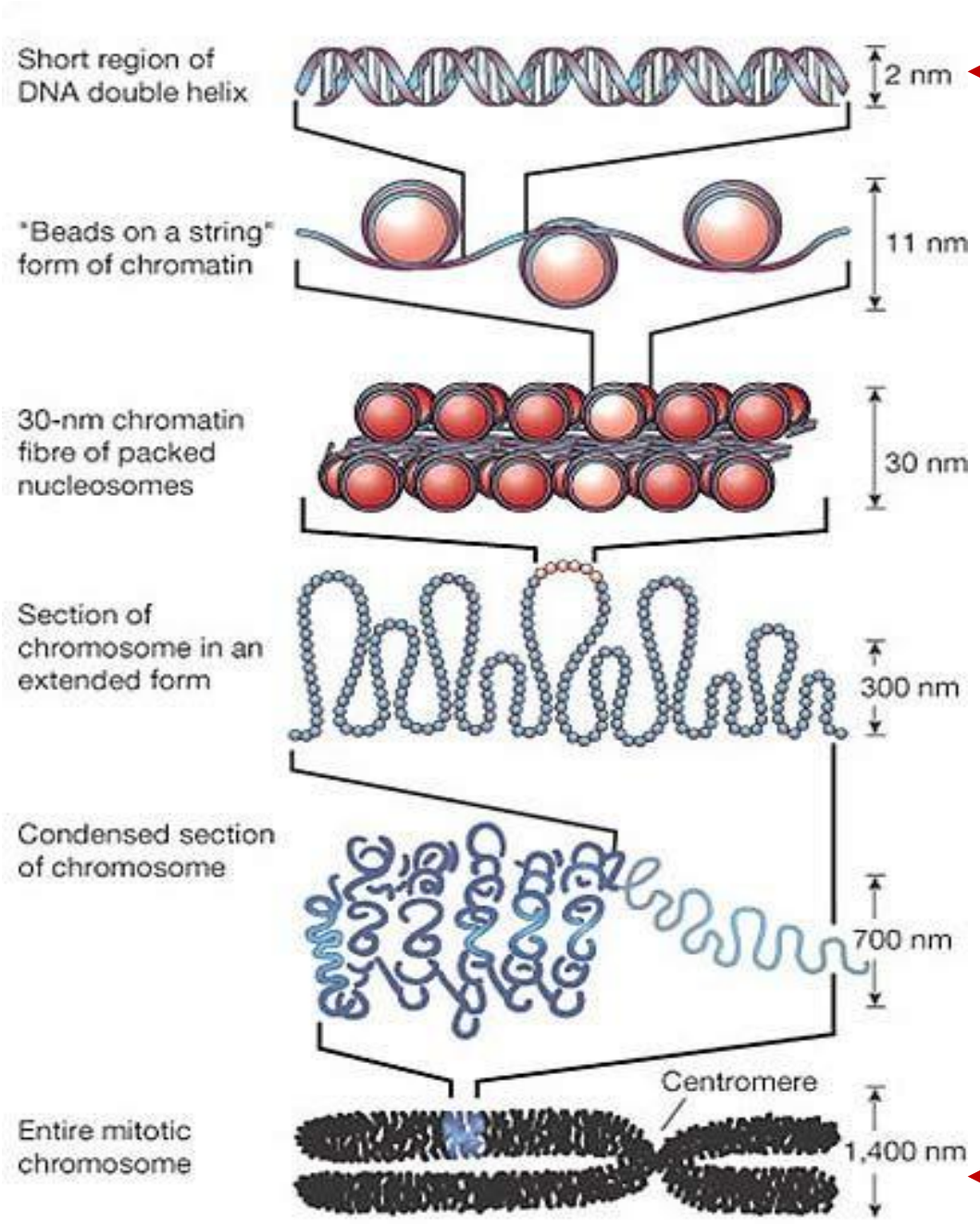
- no changes in procedure

- arose from the molecular-genetic lab

- 2 paradigm changes:

– high resolution array

– non-invasive prenatal diagnosis



molecular tests (sequencing, specific mutation detection)

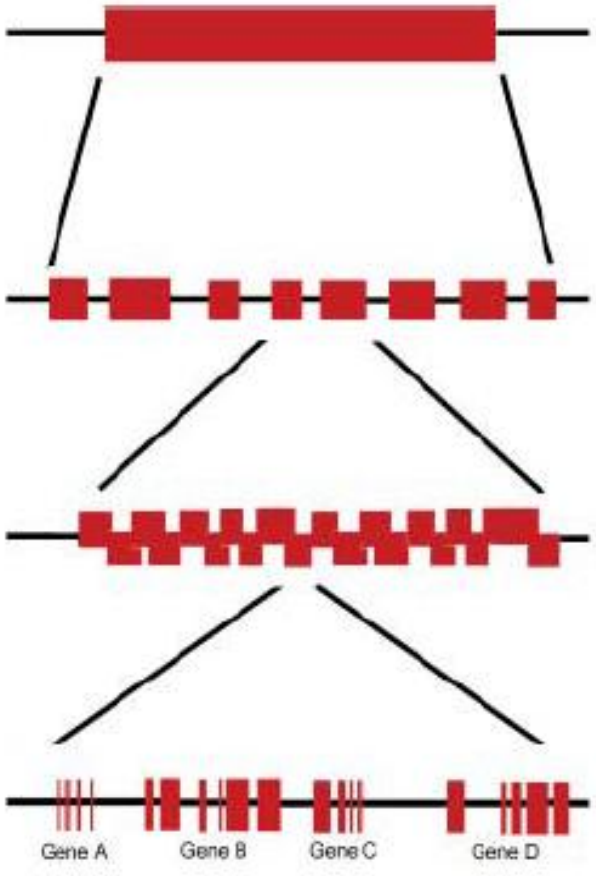
CGH arrays, SNP arrays, MLPA

Karyotyping, FISH, low-resolution CGH

Array CGH – resolution

resolution ↑ detection rate ↑

- **targeted array**
- **1 Mb** resolution (aCGH with 3,000-3,500 BAC clones)
- **10-100 kb** resolution (aCGH with 32,447 BACs/oligos)
- **exon** aCGH (all ~250,000 exons in the human genome)



Array CGH

Indications:

- mental retardation/ dysmorfic features/congenital abberations
- detection of microdeletions, microduplications

specific diagnosis not necessary

Advantages of molecular karyotyping by microarray

- higher resolution independent from cell growth and/or the possibility to produce decent metaphasis preparations

  standard karyotype: 5Mb resolution
  aCGH: 1 Mb tot ≤100 kb resolution

- direct mapping of aberrations the genome sequence
- possibility for automation and quality control procedures
- higher throughput and shorter TAT

Prenatal diagnosis based on high resolution array CGH

Category	abnormal US	AMA/ family history
clinically relevant CNV	3.9%	0%
benign CNV	8.3%	12%
unknown clinical significance	0.6%	0%
total number of cases	155	25

Coppinger J et al; Prenat Diagn. 2009 Dec;29(12):1156-66.
Whole-genome microarray analysis in prenatal specimens identifies clinically significant chromosome alterations without increase in results of unclear significance compared to targeted microarray.

Gebruik van array CGH in de prenatale diagnose

Indication	Author	Cases	Pathological CNV	Benign CNV	Unknown
US abn	Kleeman et al	50	2%	6%	
	Tyreman et al	106	9%	11%	12%
NT>3mm	Schou et al	100	0%		
US+AMA	Van den Veyver et al	300	5%	13.3%	1%

Criteria for disease loci on prenatal microarray

- significant part of the cases is caused by deletions or duplications
- disease is of clinical importance
- spectrum of the disease is known
- associated with ultrasound abnormalities

Microdeletion syndromes

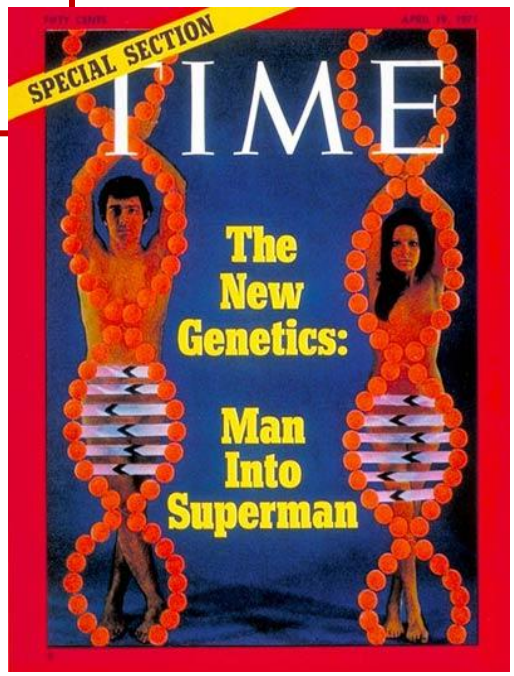
• Wolf-Hirschhorn	4p16.3
• Cri-du-Chat	5p15.2
• Williams-Beuren	7q11.23
• Beckwith-Wiedemann	11p15
• Angelman syndrome	15q11-q13
• Prader-Willi syndrome	15q11-q13
• Rubinstein-Taybi	16p13.3
• Smith-Magenis	17p11.2
• Miller-Dieker	17p13.3
• CATCH22/Di George/VCFS	22q11.2
• Kallmann	Xp22.3
• SHOX	Xp22-Yp11.3

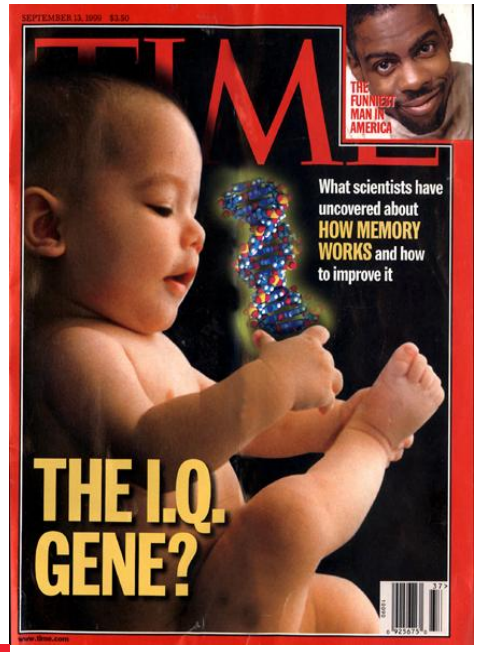
Prenatal diagnosis: the sky is the limit?

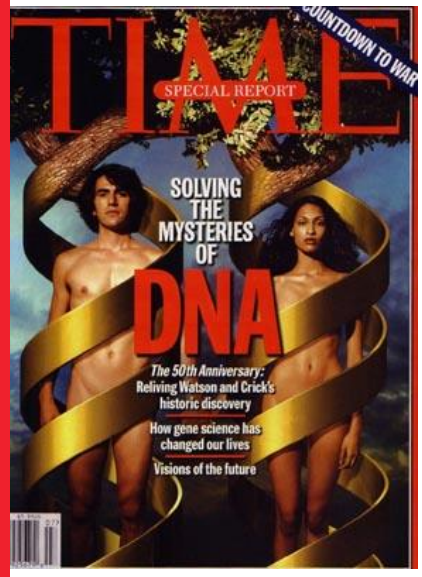
Prenatal genetic tests:
difference with non-genetic prenatal examinations?

- genetic information:
  - can predict future health-status
  - can reveal information about family members
  - can be used to discriminate/stigmatize
  - can cause psychological damage
  - identifies a disease for which there is no effective or acceptable treatment
  - complex results which are sometime hard to interpret for clinicians
- genotyping has to be done just once

Expectations versus Hype



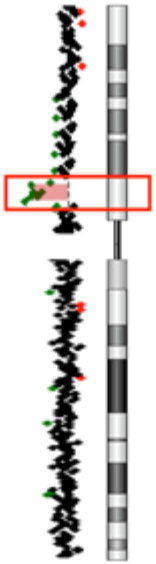




Susceptibility examination

THE DNA AGE

After DNA Diagnosis: ‘Hello, 16p11.2. Are You Just Like Me?’



Samantha Napier, 14, left, and Taygen Lane, 4, share a rare genetic mutation

Published: December 28, 2007

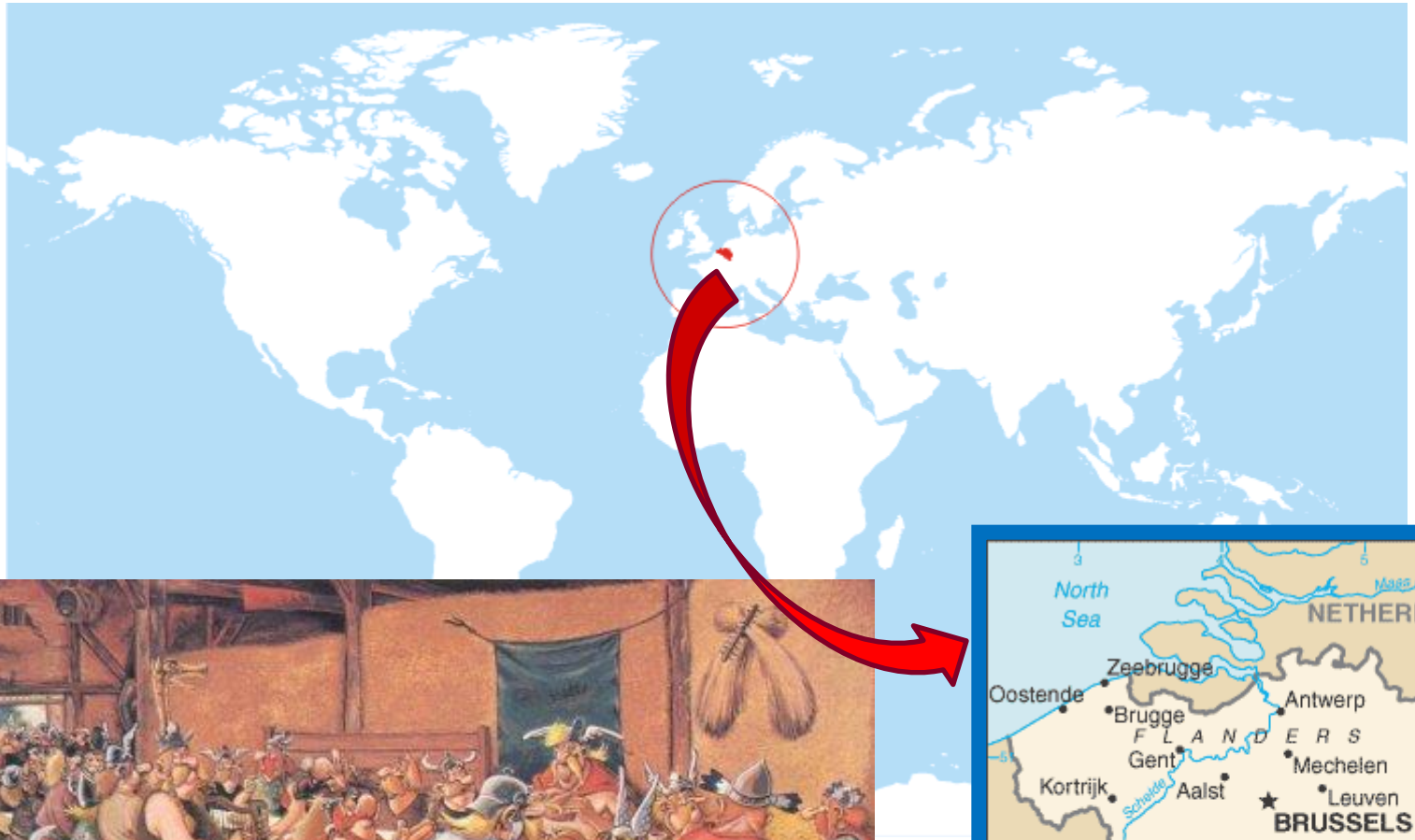
The New York Times

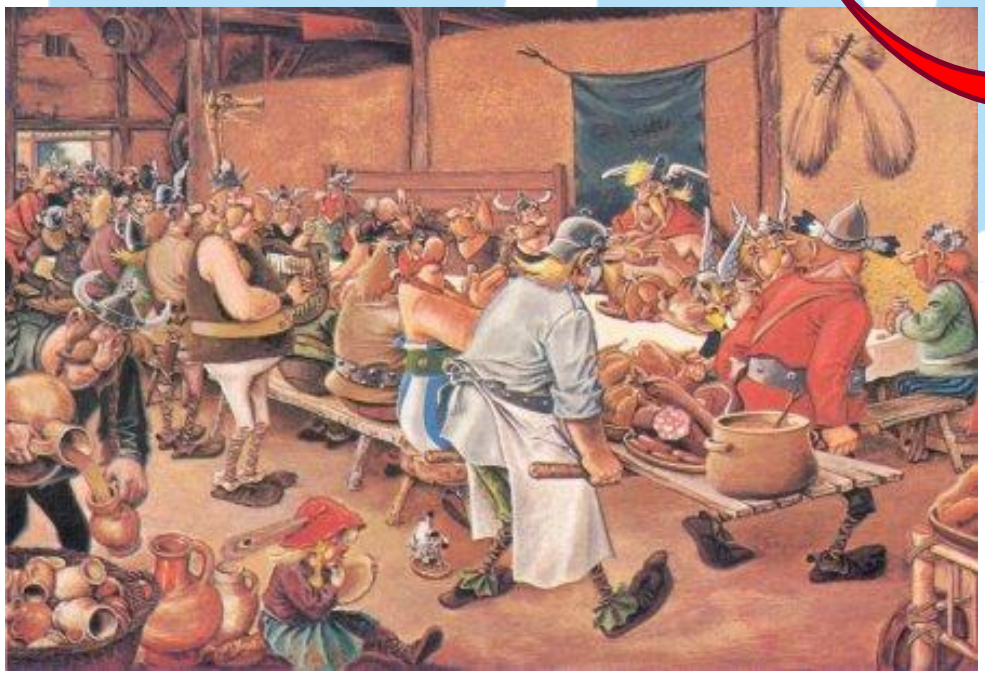
BRCA and other cancertests

Ethical questions predictive DNA test

- at what age? prenatally?
- parents request?
- for late-onset disorders (e.g. dementia)?
- only if treatment available?
- only if 100% penetrance?

Horum omnium fortissimi sunt Belgae…





Medical genetics in Belgium

- 8 Genetic Centres
  - 4 in Flanders: Antwerp, Brussels, Ghent, Leuven
  - 4 in Wallonia: Bruxelles, Liège, Louvain, Loverval

- all affiliated with a University authorized to teach medicine
- geneticists: appointed by royal decree, entitled to charge genetic analyses to the health insurance

Refund health insurance

2 PRENATALE ONDERZOEKEN

565176-565180	Combinatie van genetische testen, waaronder een (moleculair) karyotype, uitgevoerd met het oog op de detectie van een cytogenetische afwijking, op een staal van foetale oorsprong, voor het geheel der analyses (Diagnoseregel 5, 10, 20)	B 456
565191-565202	Moleculair genetische test uitgevoerd met het oog op een prenatale diagnose in het geval van het familiaal voorkomen van een genetische aandoening en/of bij foetale pathologie, op een staal van foetale oorsprong, voor het geheel der analyses (Diagnoseregel 5, 10, 20)	B 456

National implementation of prenatal array's

Belgian consensus prenatal microarray

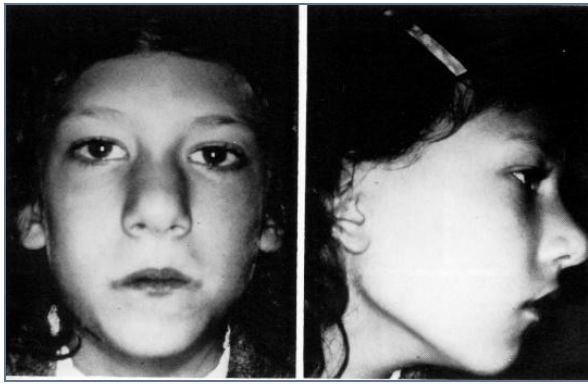
- use 60k arrays (or comparable resolution)
- always test for maternal cell contamination
- a rapid aneuploidy test is not necessary if the TAT of the array is less than one week
- always obtain a parental blood sample (can be stored)
- 6ml amniotic fluid is the minimum for direct DNA isolation to obtain reliable results on 60k array
- always have at least 1 backup flask in culture
- testing for triploidy is done by SNP array or a short tandem repeat (STR) multiplex system

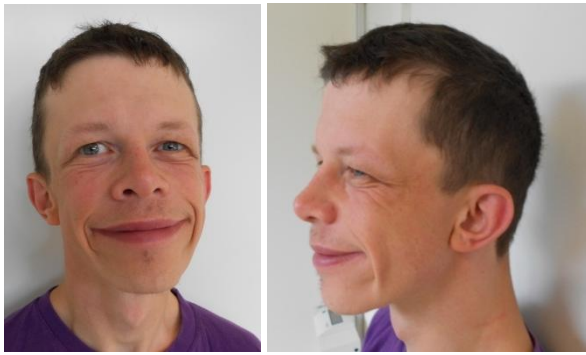
Belgian consensus prenatal microarray

- Belgian prenatal array database
- ad hoc comity
- genetic counseling and IC **without** opting out

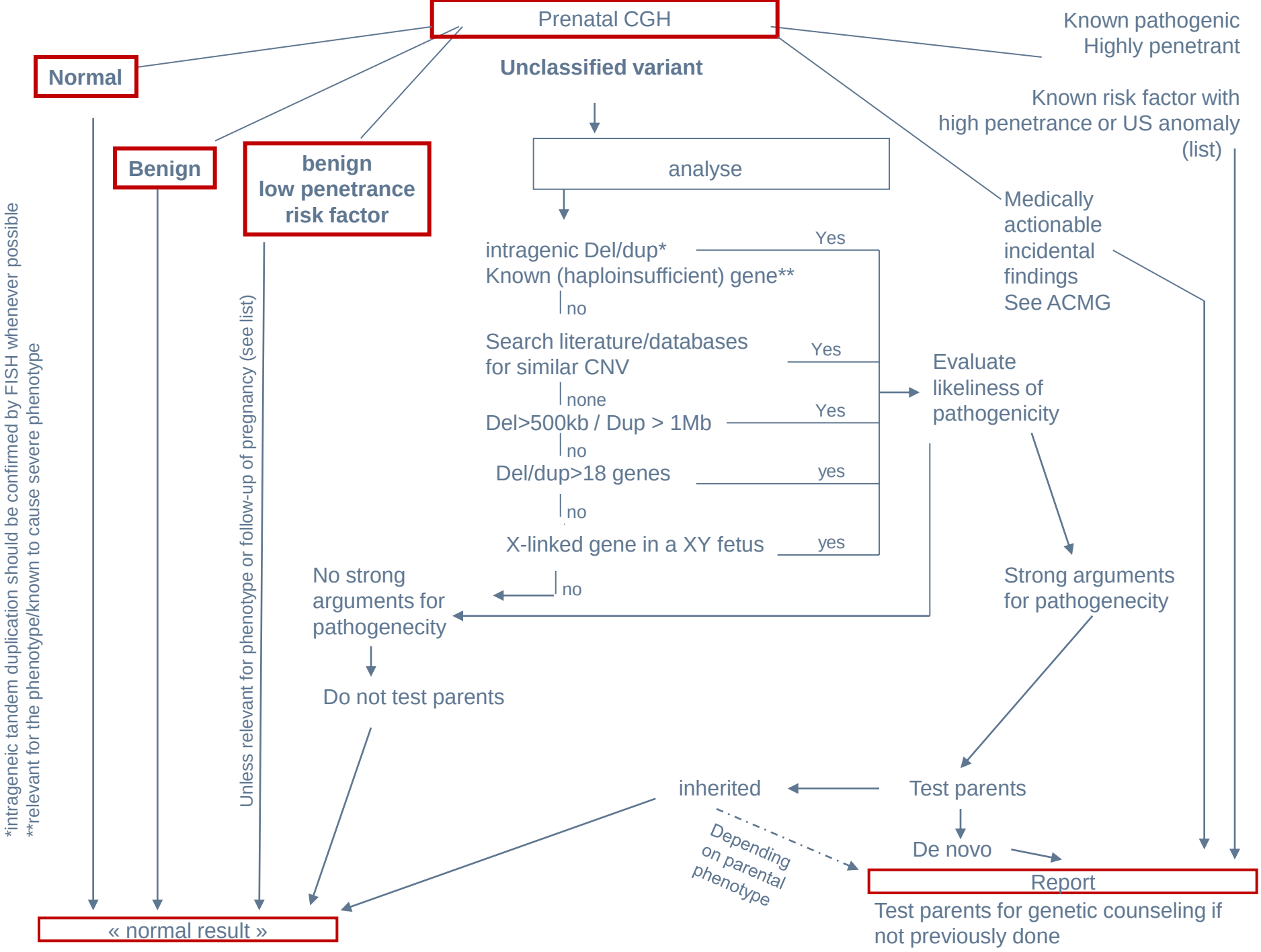
Results prenatal microarray

Classification of CNV regarding pathogenicity





- **pathogic**: - CNV **associated with phenotype** (e.g. del 22q11.2)
  - CNV **resulting in known effect** on gene function and phenotype (haploinsufficiency, e.g. Williams Σ, del 7q11.23)
- **benign**: repeatedly found in nl. population (cave: population specific => importance of Belgian database)
- **UV**: classification based on: seize, number of genes, de novo vs. inherited, overlap with reported CNV's, associated phenotype, number of published cases


Prenatal CGH
Normal
Benign
benign
low penetrance
risk factor
Unclassified variant
analyse
Known pathogenic
Highly penetrant
Known risk factor with
high penetrance or US anomaly
(list)
Medically
actionable
incidental
findings
See ACMG
intragenic Del/dup*
Known (haploinsufficient) gene**
Yes
no
Search literature/databases
for similar CNV
Yes
none
Del>500kb / Dup > 1Mb
Yes
no
Del/dup>18 genes
yes
no
X-linked gene in a XY fetus
yes
no
Evaluate
likeliness of
pathogenicity
No strong
arguments for
pathogenecity
Strong arguments
for pathogenecity
Do not test parents
Test parents
inherited
Depending
on parental
phenotype
De novo
Report
Test parents for genetic counseling if
not previously done
« normal result »
Unless relevant for phenotype or follow-up of pregnancy (see list)
*intrageneic tandem duplication should be confirmed by FISH whenever possible
**relevant for the phenotype/known to cause severe phenotype

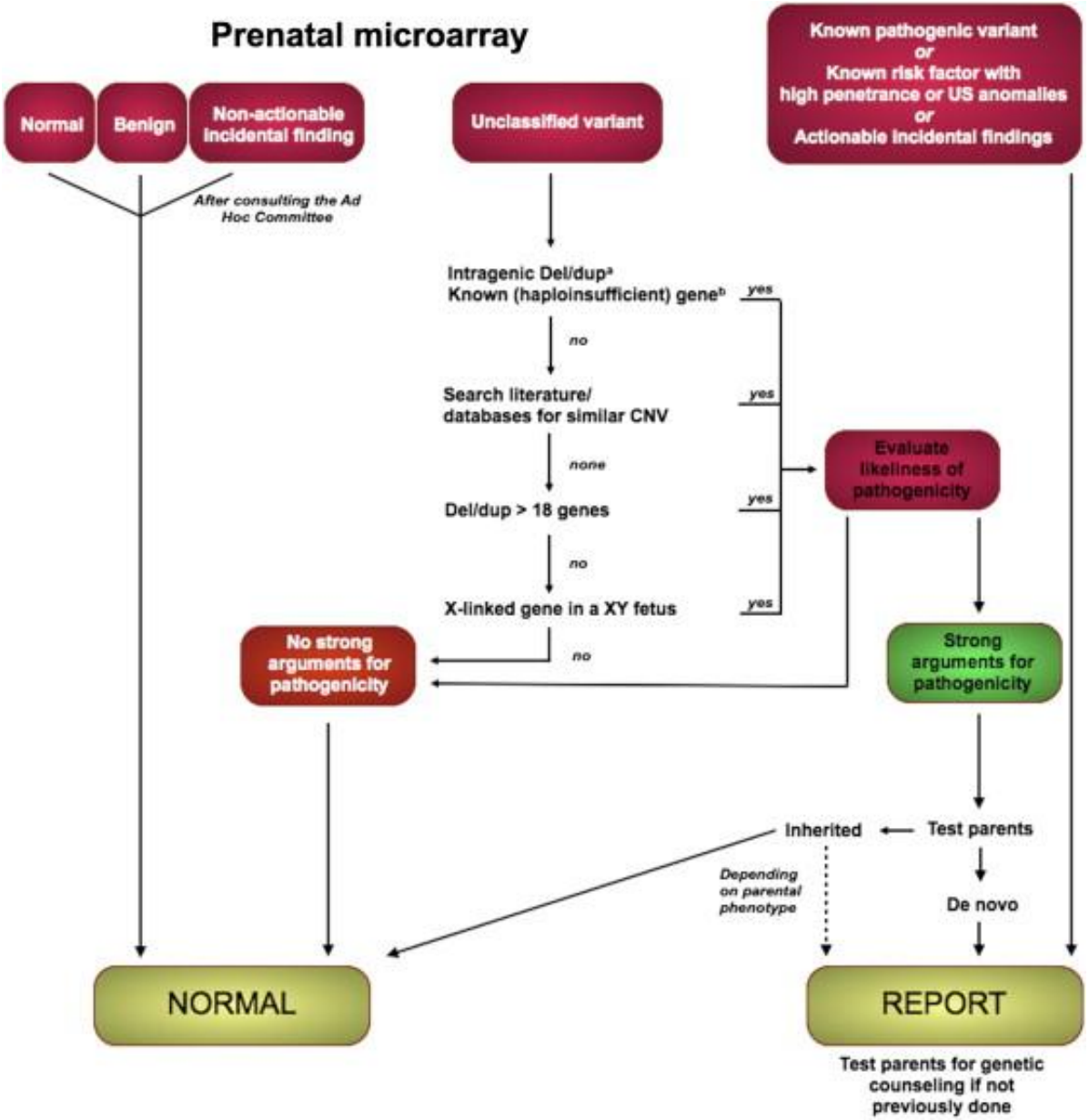


Fig. 1 Flow chart of the reporting policy presented in this paper, based on the classification of variants found with prenatal microarray. a: intragenic tandem duplications should be confirmed by FISH whenever possible; b: relevant for the phenotype or known to cause a severe phenotype

Results prenatal microarray

Susceptibility CNV's

- **in principle no communication of risk factors**
- **report in the following situation:**
  - ➢ sufficient high risk and/or
  - ➢ association of CNV with structural malformations => indication of ultrasound follow-up and/or
  - ➢ the result influences the follow-up care of the pregnancy

- Distal deletion 16p11.2	(SH2B1)	OMIM 613444
- Deletion 16p11.2	(TBX6)	OMIM 611913
- Distal deletion 1q21.1	(GJA5)	OMIM 612474
- Deletion 17q12 = RCAD	(TCF2)	OMIM 614527
- Distal duplication 1q21.1	(GJA5)	OMIM612475
- Duplication 22q11.2	(TBX1)	OMIM 608363
- Proximal deletion 1q21.1 / TAR	(HFE2)	OMIM 274000

Returning information on susceptibility CNV

chr	start	end	SIZE (kb)	del	Cases (n= 15767)	Contr (n= 8329)	*p*-value	incidence in normal population 1 out of ... cooper	incidence in normal population 1 out of ... rosenfeld	Penetrance cooper	Penetrance rosenfeld	% de novo %de novo (Girirajan 2012 NEJM)	% de novo % de novo Rosenfelt Genet Med	% second hit (Giriraja n NEJM 2012)	phenotype	morphol. anomaly	return	OMIM
chr16	28.68	29.02	340	16p11.2 distal del (SH2B1)	15	1	0.0107	8329	22246	0.94	62,4 (26,8-94,4)	30%	33,30 %	13			YES	613444
chr16	29.56	30.11	600	16p11.2 del (TBX6)	64	3	3.39E-09	2776	3707	0.96	46,8 (31,5 - 64,2)	65%	70,20 %	8		macroceph, vertebra	YES	611913
chr1	145.04	145.86		distal 1q21.1 del (GJA5 = connexin 40)	47	2	3.28E-07	4164	3707	0.96	36,9 (23-55)	20%	18%	8	schizophrenia, MR, autism ...	microceph, Cheart d?	YES	612474
chr17	31.89	33.28		RCAD (renal cysts /diabetes) (*TCF2*)	14	2	0.0484	4164	11100	0.88	34 (13,7-70)	62%	55,60 %	12		renal anomalies	YES	614527
chr1	145.04	145.86		1q21.1 dup (GJA5)	26	1	0.0002	8329	3707	0.96	29,1 (163,9-46,8)	20%	17%	7	DD	macroceph, Cheart d	YES	612475
chr22	17.40	18.67		22q11.2 dup	50	5	1.26E-05	1665	1853	0.91	21.9 (14.7-31.8)	7%	25.5	8		CLEFT chd bladder extrophy	YES	608363
chr1	144.00	144.34	340	TAR del (HFE2) or proximal 1q21,1	13	2	0.0659	4164	2246	0.87	17,3 (10,8-27,4)	nd	0%	ND	recessive	absent radius	YES	274000

chr15	28.92	30.27		15q13.3 del (CHRNA7)	42	0	1.8E-08	NVT		1.00	ND	20%		8	1% all epilepsy, ID,		no
chr16	29.56	30.11	600	16p11.2 dup (TBX6)	28	2	0.0004	4164	2224	0.93	27.4 (17.4-40,7)	20%	23.3%	15			no
chr2	110.18	110.34		2q13 del (NPHP1)	78	30	0.0813 not significant	277	ND	0.72	34,4 (13-70)		ND	nd	recessive	nephronopht	NO
chr17	31.89	33.28		17q12 dup	18	3	0.0361 (marginally significant)	2776	4000	0.86	21.1 (10.6-39.5)	15%	22.2%	14			NO
chr1	144.00	144.34		1q21.1 dup (HFE2)	25	6	0.0511 (not significant, large numbers)	1388	2246	0.81	17,3 (10,8-27,4)		0%	ND		NONE	NO
chr16	15.41	16.20		16p13.11 del (MYH11)	18	3	0.0361	2776	1853	0.86	13,1 (7,91-21,3)	22%	21,70%	10		none	NO
chr16	21.85	22.37	520	16p12.1 del (EEF2K, CDR2)	37	3	0.0001	2776	1390	0.93	12,3 (7,91-18,8)	5%	3,60%	22			NO
chr16	28.68	29.02	340	16p11.2 distal dup (SH2B1)	14	2	0.0484	4164	2224	0.88	11.2 (6.26-19.8)	11%	12.5%	30			NO
chr15	20.35	20.64		15q11.2 del (NIPA1)	94	19	2.13E-05	438	264	0.83	10,4 (8,45-12,7)	5%	0	15		CHD??	NO
chr13	19.71	19.91		13q12 dup (CRYL1)	4	0	0.1833	NVT	ND	1.00	ND	ND	ND	ND			NO
chr2	110.18	110.34		2q13 dup (NPHP1)	118	32	0.0003	260	ND	0.79	ND	ND	ND	ND			NO
chr15	20.35	20.64		15q11.2 dup (NIPA1)	64	36	0.66 (not signific)	231	ND	0.64	ND	ND	ND	ND			NO
chr15	28.92	30.27		15q13.3 dup (CHRNA7)	20	3	0.0200	2776	ND	0.87	ND	0%	ND	12%			NO
chr16	15.41	16.20		16p13.11 dup (MYH11)	24	10	0.3315 (not significant)	832	nd	0.71	ND	10%	ND	10%		AORTA DILATATION	no
chr16	21.85	22.37	520	16p12.1 dup (EEF2K, CDR2)	4	1	0.4368 (not significant)	8329	ND	0.80	ND	ND	ND	ND			NO
chr13	19.71	19.91		13q12 del (CRYL1)	14	12	0.9240 (not significant)	694		0.54	ND		ND	nd		none	NO

Characteristics of susceptibility CNV's that are communicated

CNV	Size	Gene	OMIM	p-value[a]	Ctrls (1/) *,**	Penetrance **	% de novo **,***	% 2nd hit***	Neurodevelop-mental phenotype	Ultrasound anomaly	References
Distal del 1q21.1	1.35 Mb	GJA5	613474	3.28E-07	3821	36.9 (23-55)	18-20%	8	DD, ID, ASD, E, ADHD	Microcephaly, CHD, Eye	Haldeman-Englert et al., 2011
Distal dup 1q21.1	1.35 Mb	GJA5	612475	0.0002	4367	29.1 (163.9-46.8)	16.7-20%	7	DD, ID, ASD, SCZ	Macrocephaly, CHD,Eye	Haldeman-Englert et al., 2011
Proximal del 1q21.1	200 kb	HFE2	274000	0.0659	10191	17.3 (10.8-27.4)	0%	ND		Absent radius (TAR - recessive phenotype)	Toriello et al., 2012
Distal del 16p11.2	220 kb	SH2B1	613444	0.0107	15287	62.4 (26.8-94.4)	30-33.3%	13	DD, ID		Bachmann et al., 2010
Del 16p11.2	550 kb	TBX6	611913	3.39E-09	3397	46.8 (31.5 - 64.2)	65-70.2%	8	DD, ID, ASD, E	(CHD)	Wat et al., 2011; Miller et al. 2011
Del 17q12	1.4 Mb	TCF2	614527	0.0484	7643	34 (13.7-70)	55.6-62%	12	DD, ID, ASD, (SCZ)	CAKUT	Moreno-De-Luca et al., 2010; Mefford et al., 2007; Decramer et al., 2007; Ulinski et al., 2006
Dup 22q11.2	1.5-Mb / 3Mb	TBX1	608363	1.26E-05	1798	21.9 (14.7-31.8)	7-25.5%	8	DD, ID	Bladder extrophy, (CHD), (CP)	Firth et al., 2009; Draaken et al., 2010; Lundin et al., 2010; Portnoï et al., 2009

References

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Results prenatal microarray

Incidental findings

- **late-onset with clinical suitability**: report because of health-benefit (e.g. deletion tumor suppressor gene)
- **late-onset without therapeutic possibilities:** attending geneticist decides after consultancy with ad hoc comity (e.g. duplication APP)
- **carrier X-linked recessive disorder**: both de novo and inherited will be reported
- **carrier AR:** not reported unless frequent disorder (carrier frequency >1/50) and analysis is possible in Belgium (CF, SMA, connexine 26)

Lazarin et al. Genet Med. 2013 Mar;15(3):178-86. An empirical estimate of carrier frequencies for 400+ causal Mendelian variants: results from an ethnically diverse clinical sample of 23,453 individuals)

National implementation of prenatal array's

Conclusion

- from may 2013: karyotyping prenatally is replaced by microarray in Belgium

- communication of CNV depends on different factors, in dubio: national ad hoc comity

- counseling of couples regarding the diagnostic offer during pregnancy is a sine qua non