染色体芯片在产前诊断中的应用

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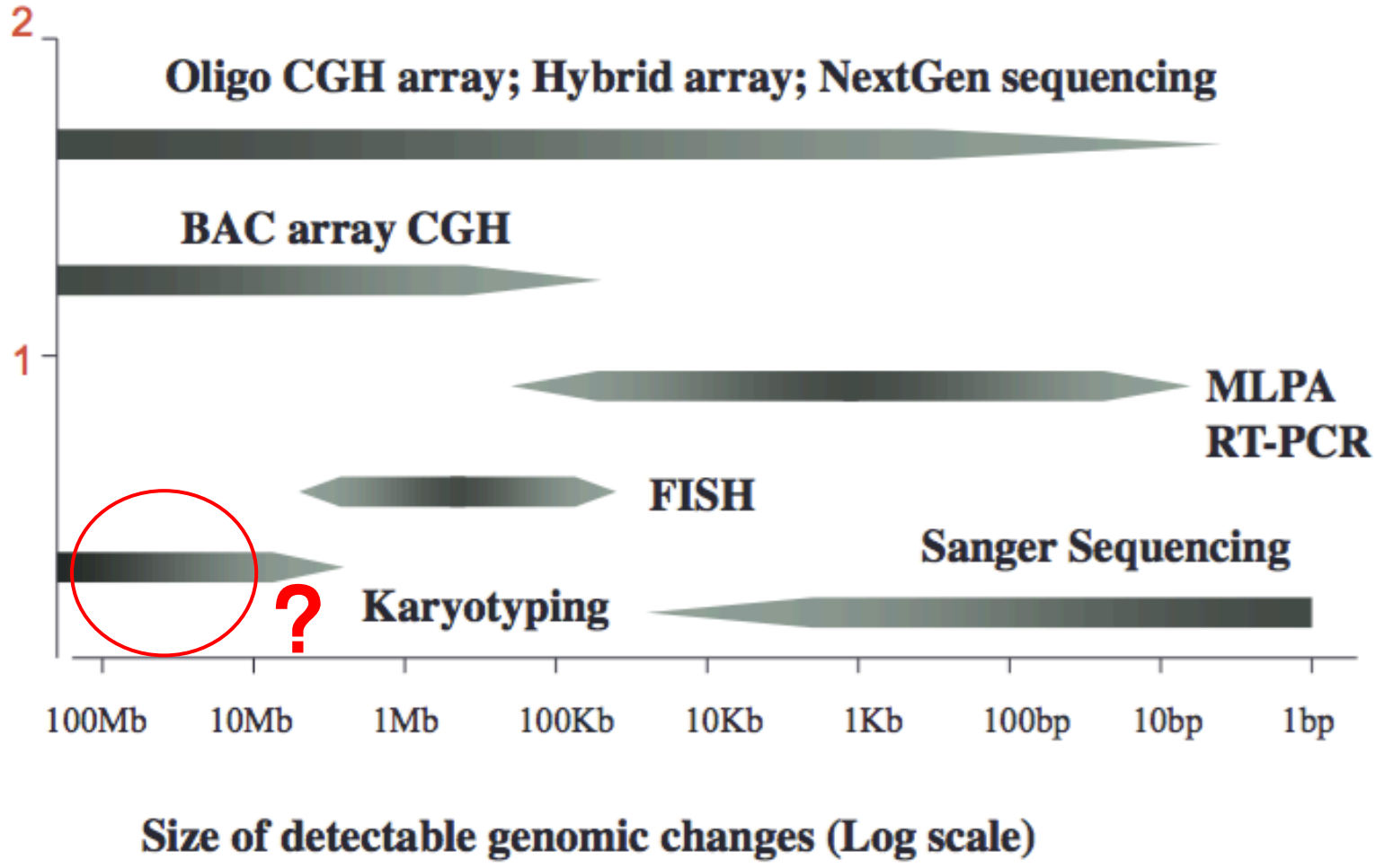
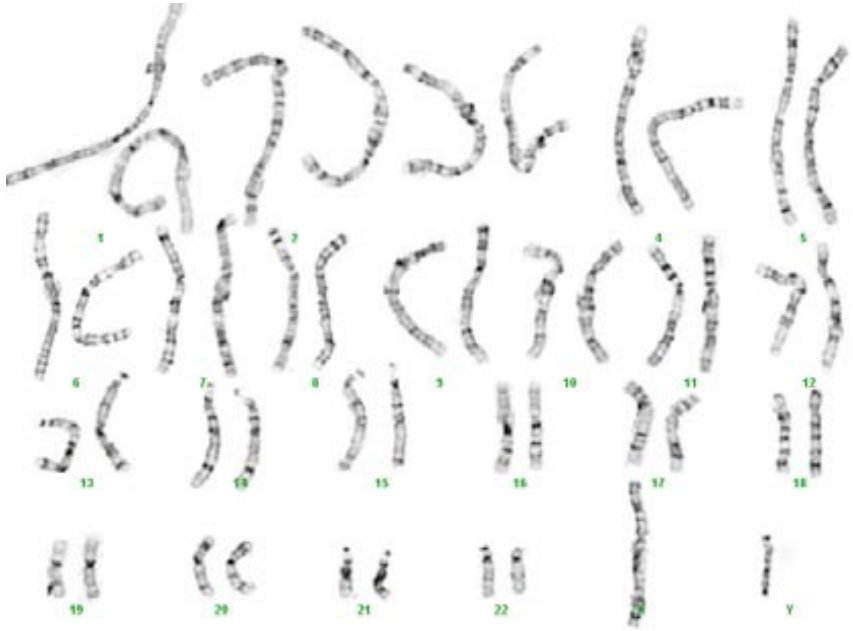
微信扫描二维码可下载本次幻灯文件

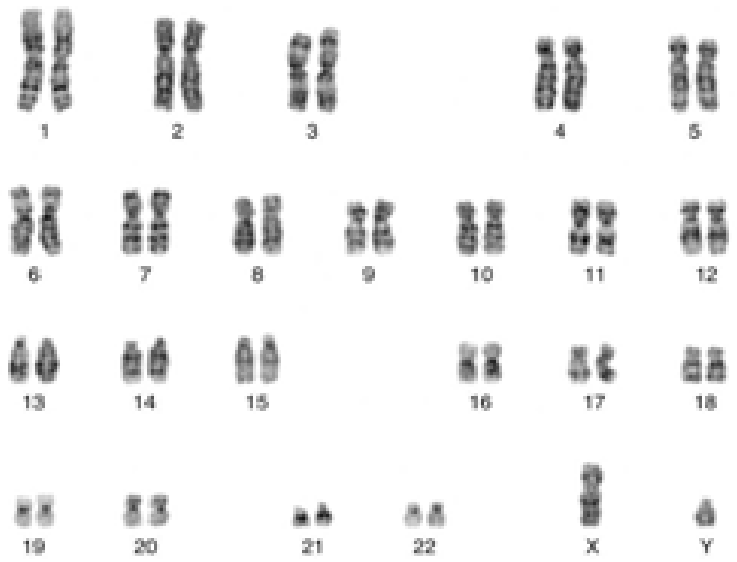
人类基因突变谱系与检测方法



普通/高分辨核型分析

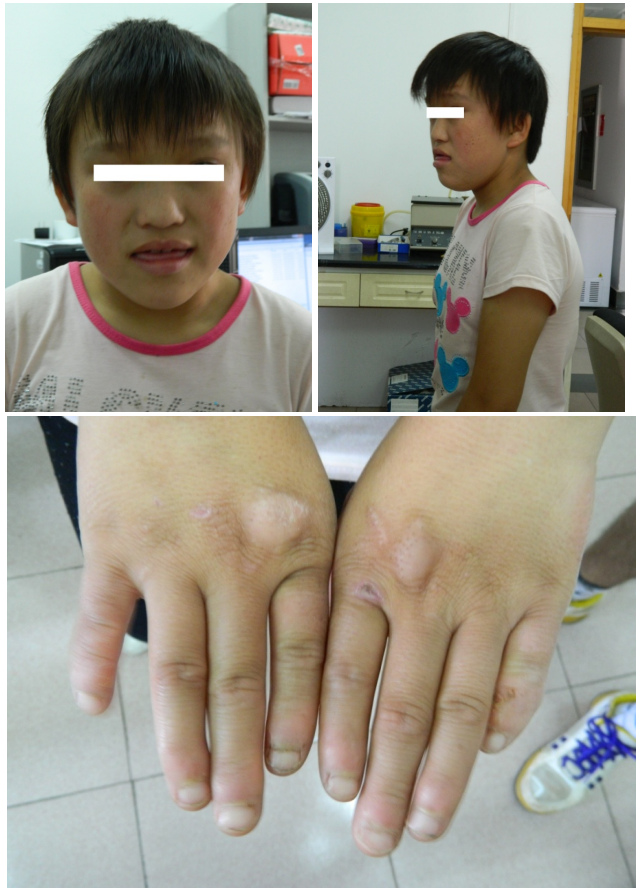


高分辨G显带650条带以上



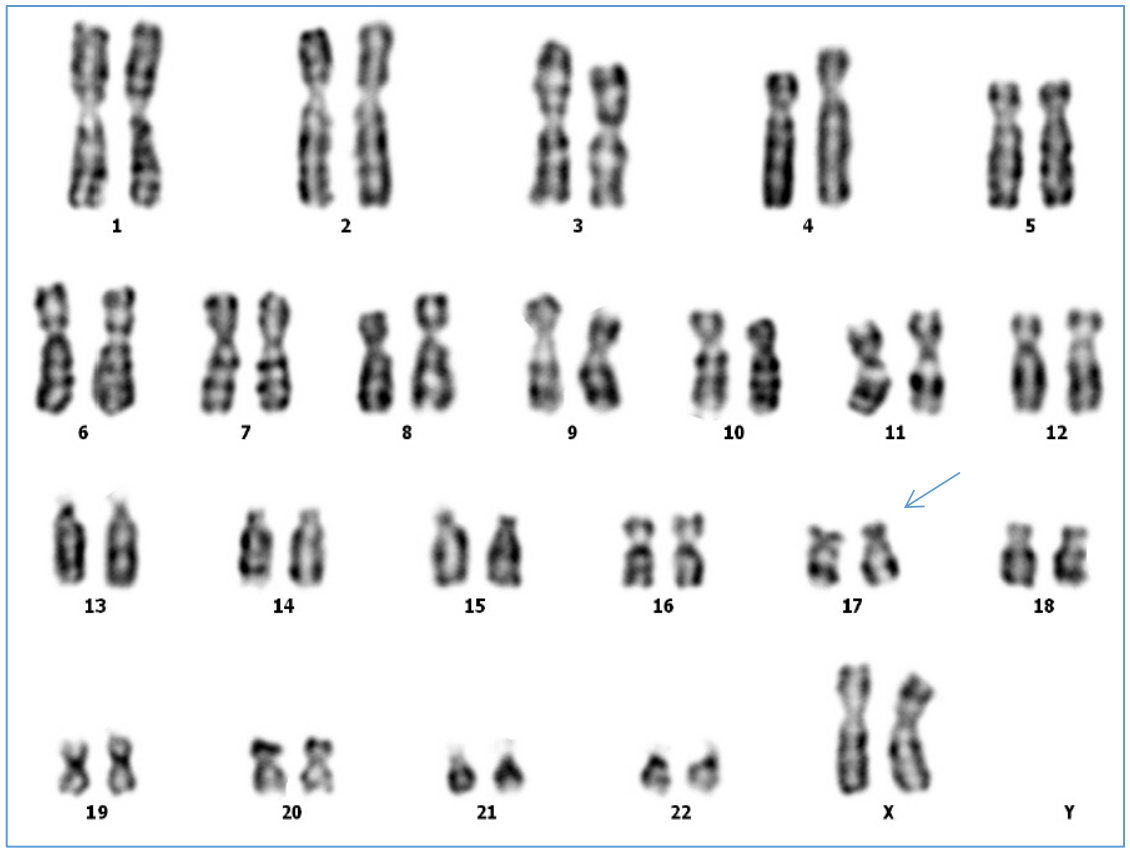
常规G显带340条带

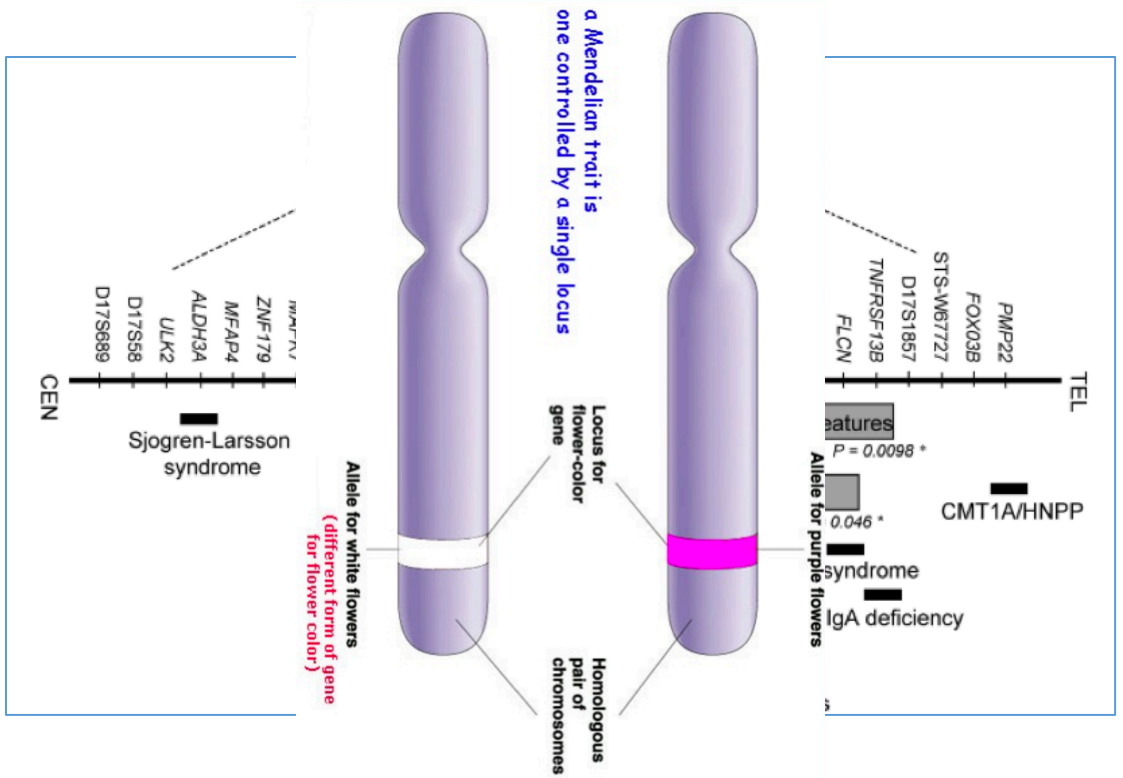
遗传诊断 — 核型分析就够了吗?



- 女性患儿15岁，身高1.44米，智力低下，语言能力弱，能回答姓名等简单问题，容易兴奋，在诊室内又唱又跳
- 面部异常:外眦上斜,巨舌,外吐
- 睡眠:现在每天仅仅睡眠2-3小时，时睡时醒
- 手:双手有咬伤伤痕。暴躁时抓人打人,自己抓头发、抓耳,指甲损伤严重。

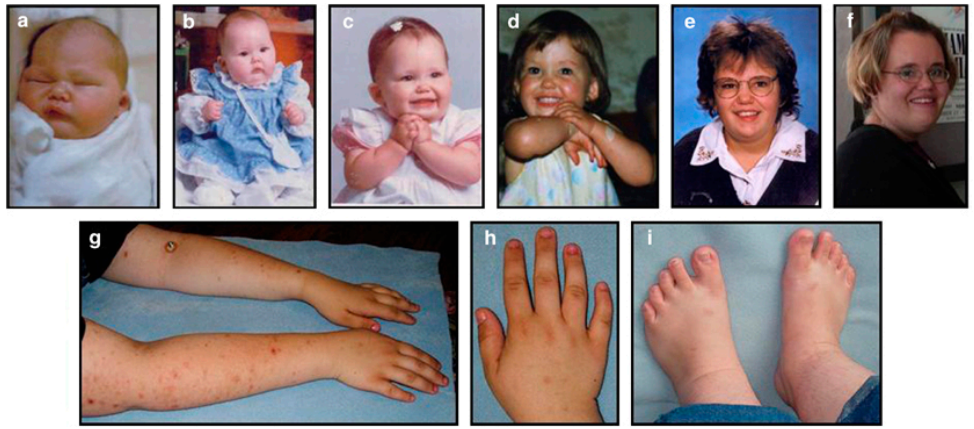
G3P3，该患儿为长女，系足月顺产，出生后无抢救史，无癫痫发作史，喂养正常，婴儿期无明显暴躁迹象





Smith-Magenis综合征(SMS) -17p11.2

Smith-Magenis综合征(SMS) -17p11.2

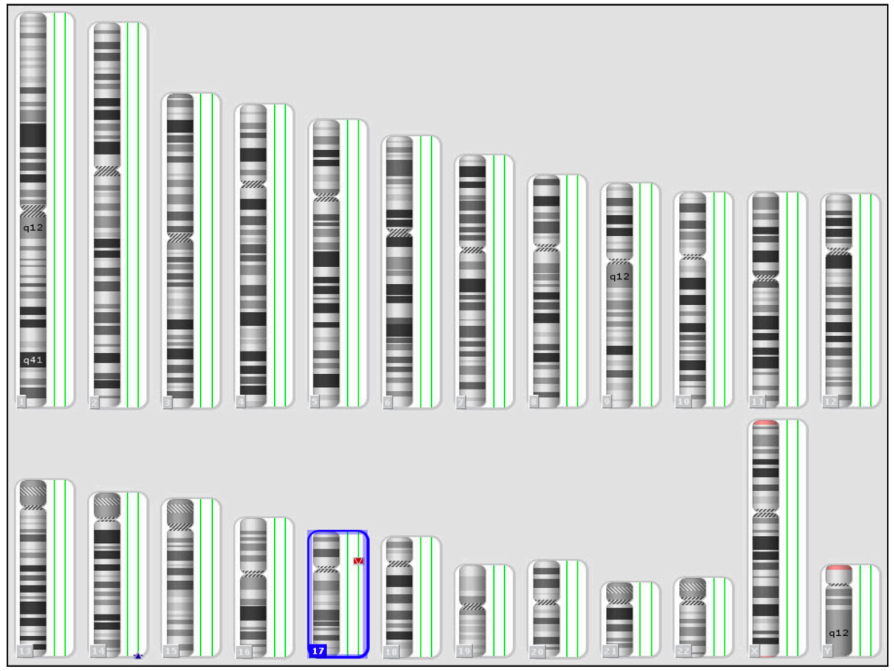


Signs and Symptoms	Approximate number of patients (when available)
Abnormality of the tracheobronchial system	Very frequent (present in 80%-99% of cases)
Anxiety	Very frequent (present in 80%-99% of cases)
Attention deficit hyperactivity disorder	Very frequent (present in 80%-99% of cases)
Brachycephaly	Very frequent (present in 80%-99% of cases)
Brachydactyly	Very frequent (present in 80%-99% of cases)
Broad forehead	Very frequent (present in 80%-99% of cases)
Corticospinal tract hypoplasia	Very frequent (present in 80%-99% of cases)
Deeply set eye	Very frequent (present in 80%-99% of cases)
Delayed eruption of primary teeth	Very frequent (present in 80%-99% of cases)
Delayed speech and language development	Very frequent (present in 80%-99% of cases)

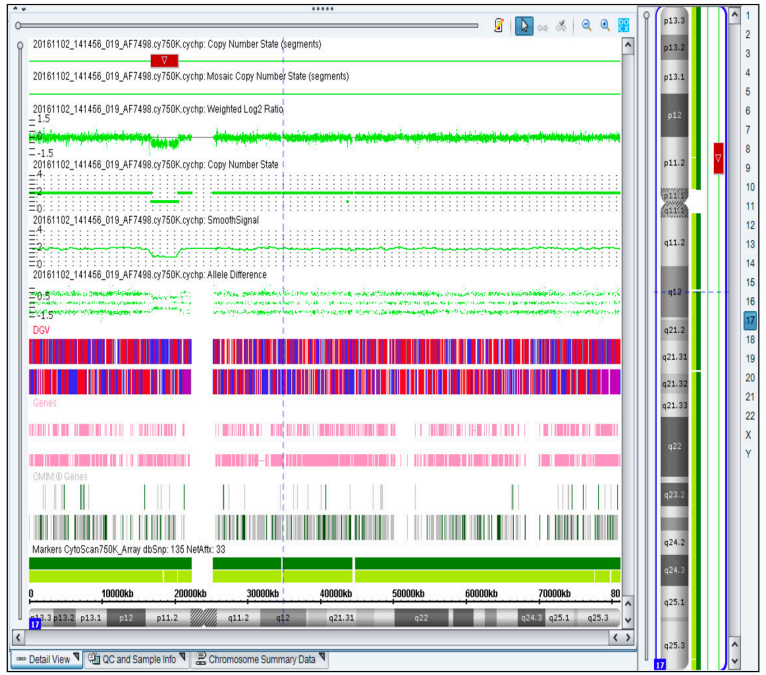
- 较常见的微缺失综合征，发生率1/25000
- 17p11.2缺失导致
- 产前临床表现：无报道
- 产后临床表现:：发育迟缓、智力迟滞、行为异常等
  - 小头畸形，联眉，内眦赘皮，
  - 入睡困难，易激惹、注意力低下，自残行为，痛阈低下，剔甲癖等

J Med Genet,1999,36:394-397
BMC Medical Genetics,2010,11:142-146

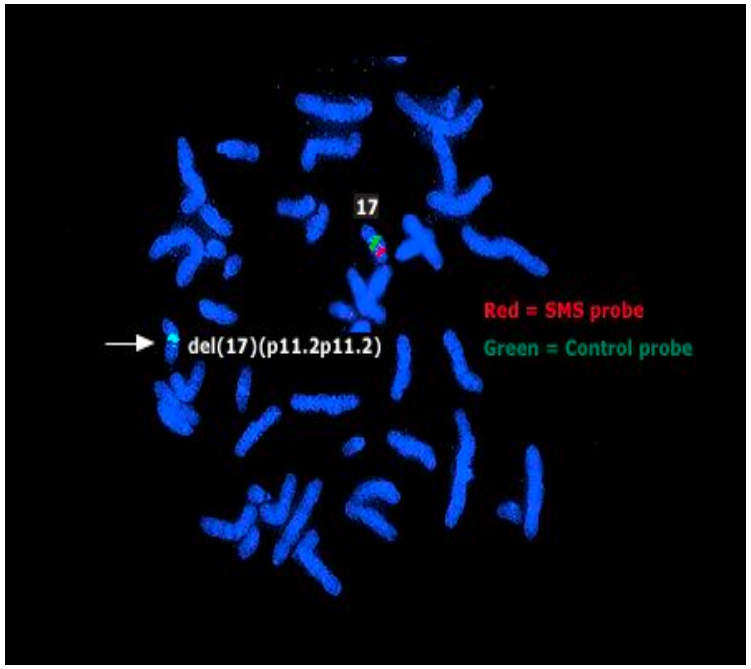
如何诊断 — 染色体芯片（Microarray）结合荧光原位杂交技术（FISH）



染色体芯片发现缺失位点



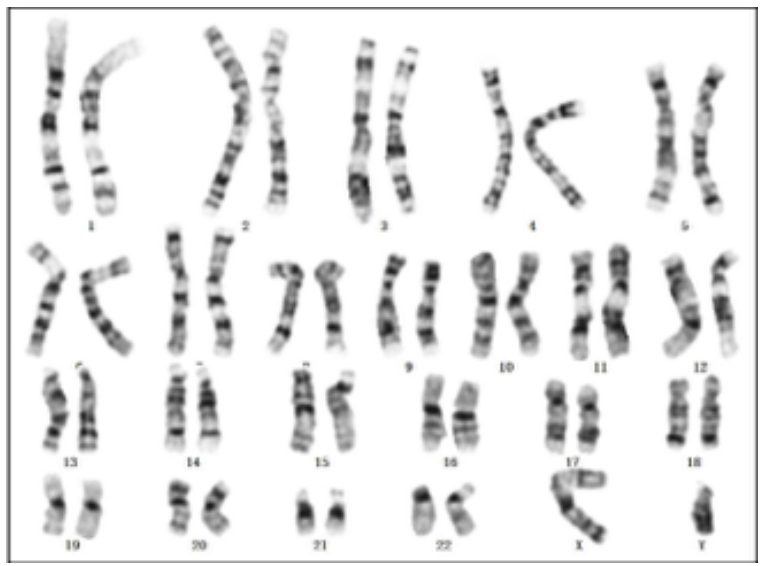
染色体缺失详图

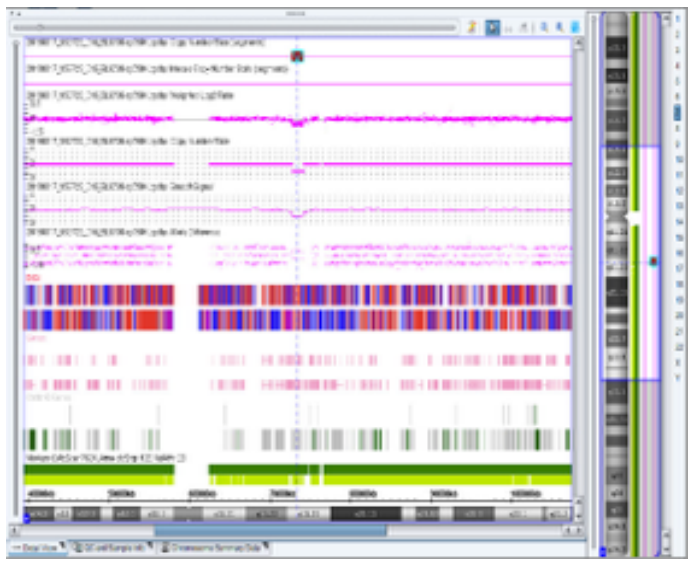


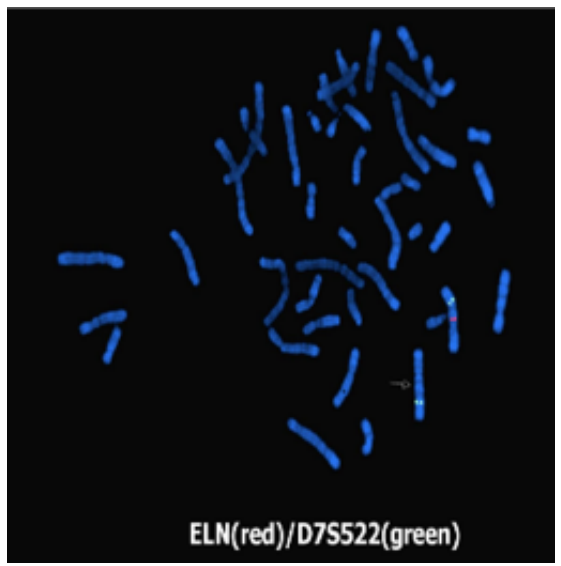
FISH验证缺失的存在

5岁男孩，智力和体格发育迟缓，幼年时做过心脏室缺修补术，怀疑是某种染色体病

46,XY.ish del(7)(q11.23q11.23)(ELN-)dn　Williams-Beuren综合征







46，XY

7q11.23(72,749,941-74,154,209)缺失1.4M

ish del(7)(q11.23q11.23)(ELN-)

Williams-Beuren综合征(WBS)-7q11.23缺失

- 产前临床表现:IUGR
- 产后临床表现:
  - 70%伴有心血管畸形（主动脉流出道狭窄），特殊面容（嘴唇厚，鼻孔前倾，长人中）、智力迟滞、发育迟缓、特殊性格，皮肤早熟等
  - 婴儿期常有腹痛发作，高血钙表现
  - 有突出的社交性，表现为喜欢外出，交流过度频繁，待人过份友好和善
  - 中枢神经系统和骨骼肌肉系统的发育迟缓
- 该综合征不能被常规或高分辨核型分析发现

J Med Genet, 2003;40:526-530
Arch Dis Child 1999;81:198-200
Arq Bras Cardiol,2003;81(5):468-73
Am J Human Genet,1999;64,000
J Med Genet 2007;44:2 136-143

Table 1: Description of the aneuploidies and microdeletion syndromes included in the Prenatal BoBS kit.

Syndrome	Frequency of occurrence	Lifespan	Mental retardation	Severe medical symptoms
Down syndrome (21)	1/750–800	50 years	Mild to moderate	−/+
Patau syndrome (13)	1/6,000	4 days	Severe	++
Edwards syndrome (18)	1/10,000	2.5 days	Severe	++
Triple X syndrome (XXX)	1/1,000	Normal	No	−
Klinefelter syndrome (XXY)	1/500–1,000	Normal	No	−
XYY syndrome (XYY)	1/1,000	Normal	No	−
Turner syndrome (X0)	1/2,500	Slightly reduced	Mild to moderate	−/+
Wolf-Hirschhorn (4p16, 3)	1/50,000	Limited	Moderate to severe	+
Cry du Chat (5p15, 3-p15, 2)	1/15,000–50,000	Normal	Moderate to severe	−/+
Williams-Beuren (7q11, 2)	1/7,500–20,000	Reduced	Mild to moderate	−/+
Langer-Giedion (8q23-q24)	unknown	Normal	Mild to severe	−/+
Prader-Willi (15q11-q12)	1/10,000–30,000	Normal	Mild	−/+
Angelman (15q11-q12)	1/12,000–25,000	Normal	Severe	−/+
Miller-Dieker (17p13, 3)	1/100,000–300,000	Reduced	Profound	−/+
Smith-Magenis (17p11, 2)	1/25,000–50,000	No data	Mild to moderate	−/+
DiGeorge (10p14)	1/4,000–5,000	Reduced	Mild to moderate	+
DiGeorge (22q11, 2)	1/2,000–4,000	Reduced	Mild to moderate	+

The severity and type of the symptoms are represented from − (in cases where symptoms range from none to mild) to ++ (for those ranging from moderate to severe). The information in this table was adapted from the following resources: http://www.orpha.net, http://www.nlm.nih.gov, and http://www.rarechromo.org.

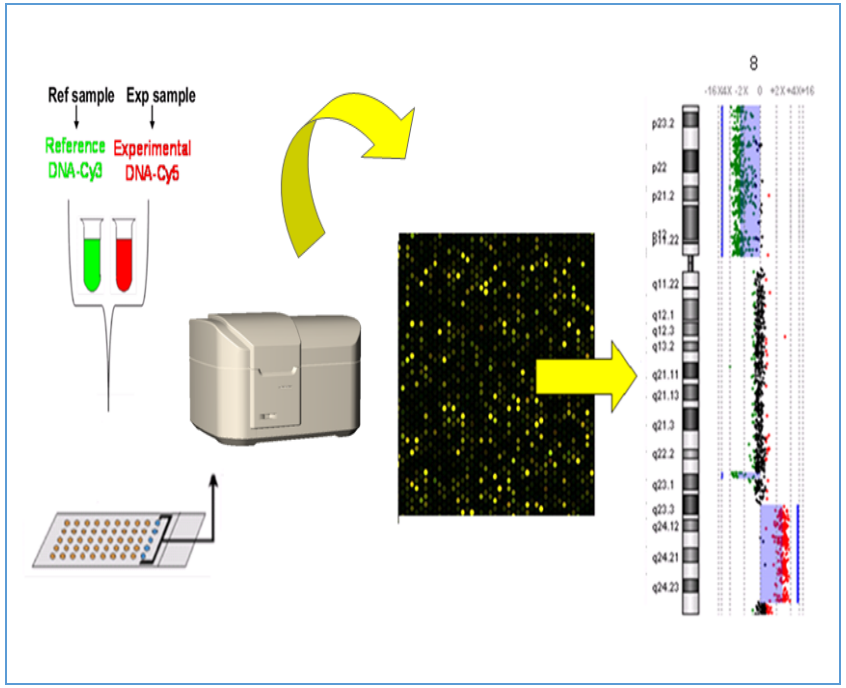
9种常见微缺失综合征发生率合计1/1600

常见的微缺失综合征

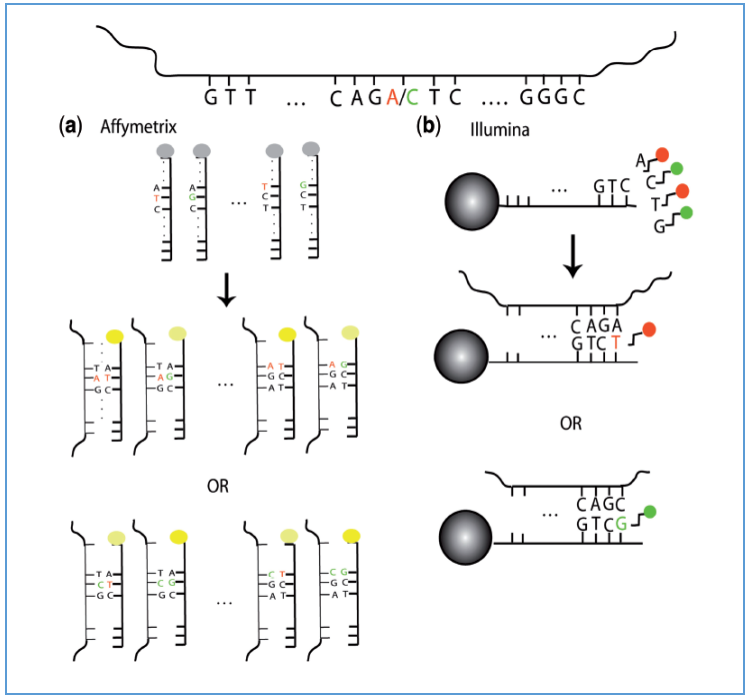
疾病名称	检测区域	发病率
Wolf-Hirschhorn syndrome	4p16.3	1/50,000
Cri du Chat syndrome	5p15.3-p15.2	1/15,000 – 1/50,000
Williams-Beuren syndrome	7q11.2	1/7,500 – 1/20,000
Langer-Giedion syndrome	8q23-q24	1/200,000
Prader-Willi / Angelman syndrome	15q11.2-q13	1/10,000 – 1/25,000
Miller-Dieker syndrome	17p13.3	1/100,000 – 1/30,000
Smith-Magenis syndrome	17p11.2	1/15,000 – 1 / 25,000
DiGeorge syndrome	22q11.2	1/4,000
DiGeorge II	10p14	1/6,000

染色体微阵列基因芯片（chromosomal microarray analysis，CMA）

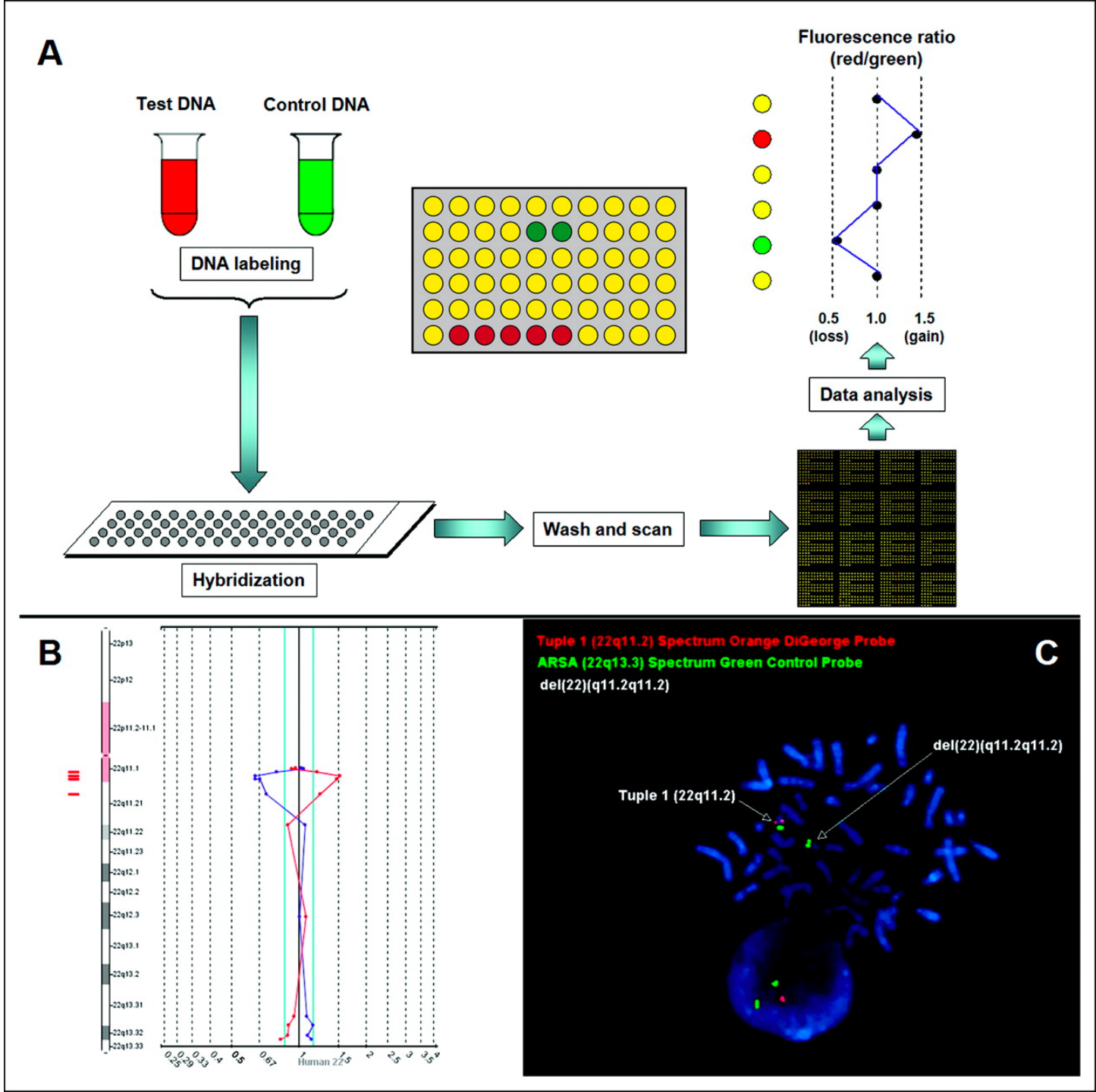
基于CGH的平台



基于 SNP的平台



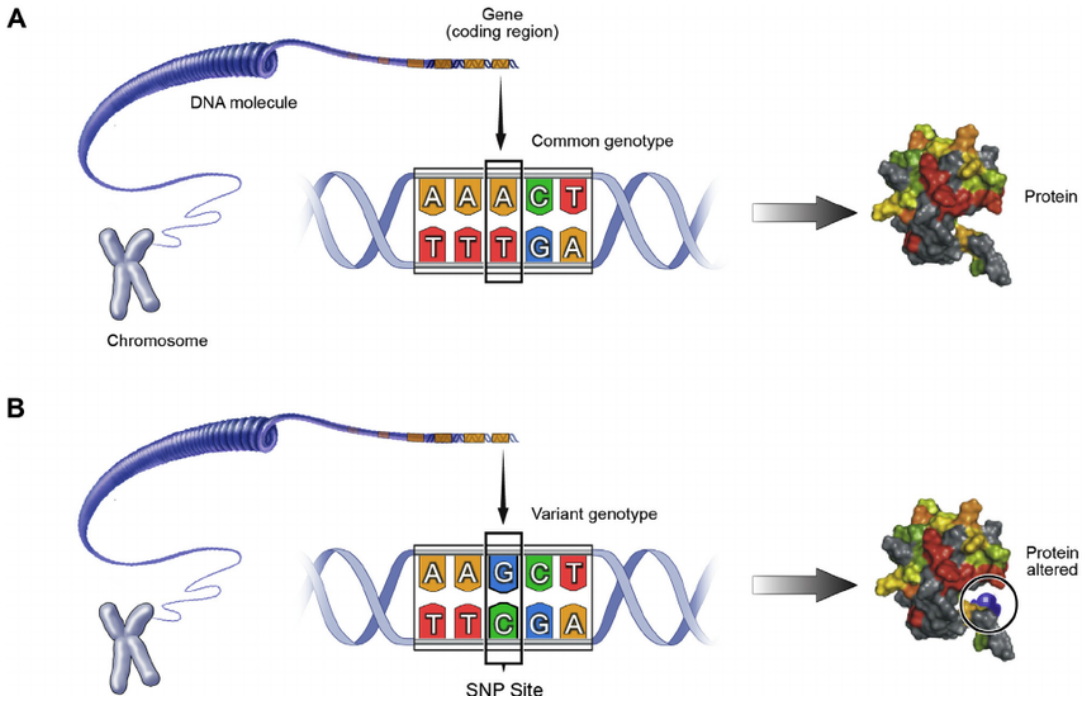
Array CGH原理

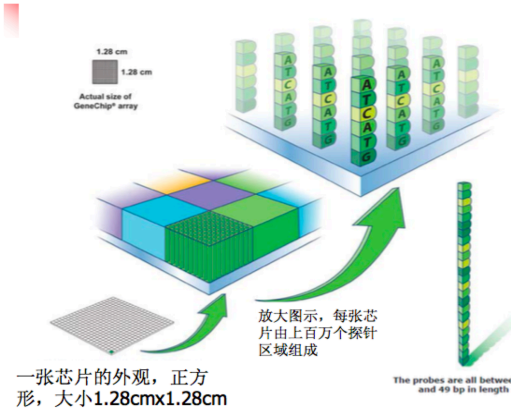

A
Test DNA
Control DNA
DNA labeling
Hybridization
Wash and scan
Data analysis
Fluorescence ratio
(red/green)
0.5
(loss)
1.0
1.5
(gain)
B
C
Tuple 1 (22q11.2) Spectrum Orange DiGeorge Probe
ARSA (22q13.3) Spectrum Green Control Probe
del(22)(q11.2q11.2)
Tuple 1 (22q11.2)
del(22)(q11.2q11.2)

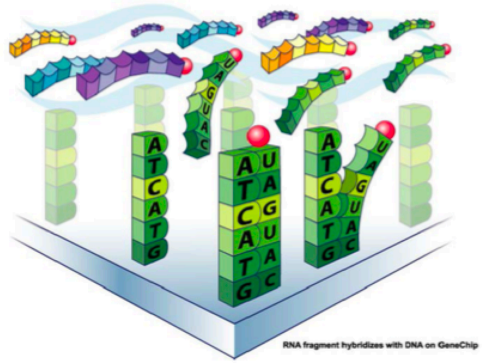
SNP Array原理

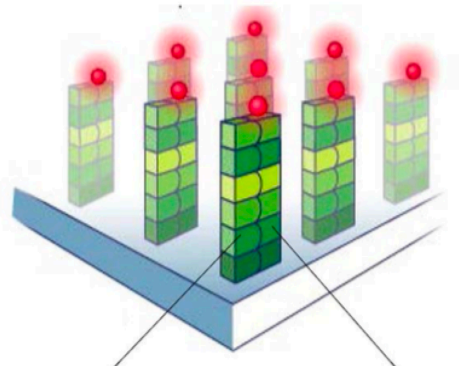
核型分析的分辨率　10Mb以上

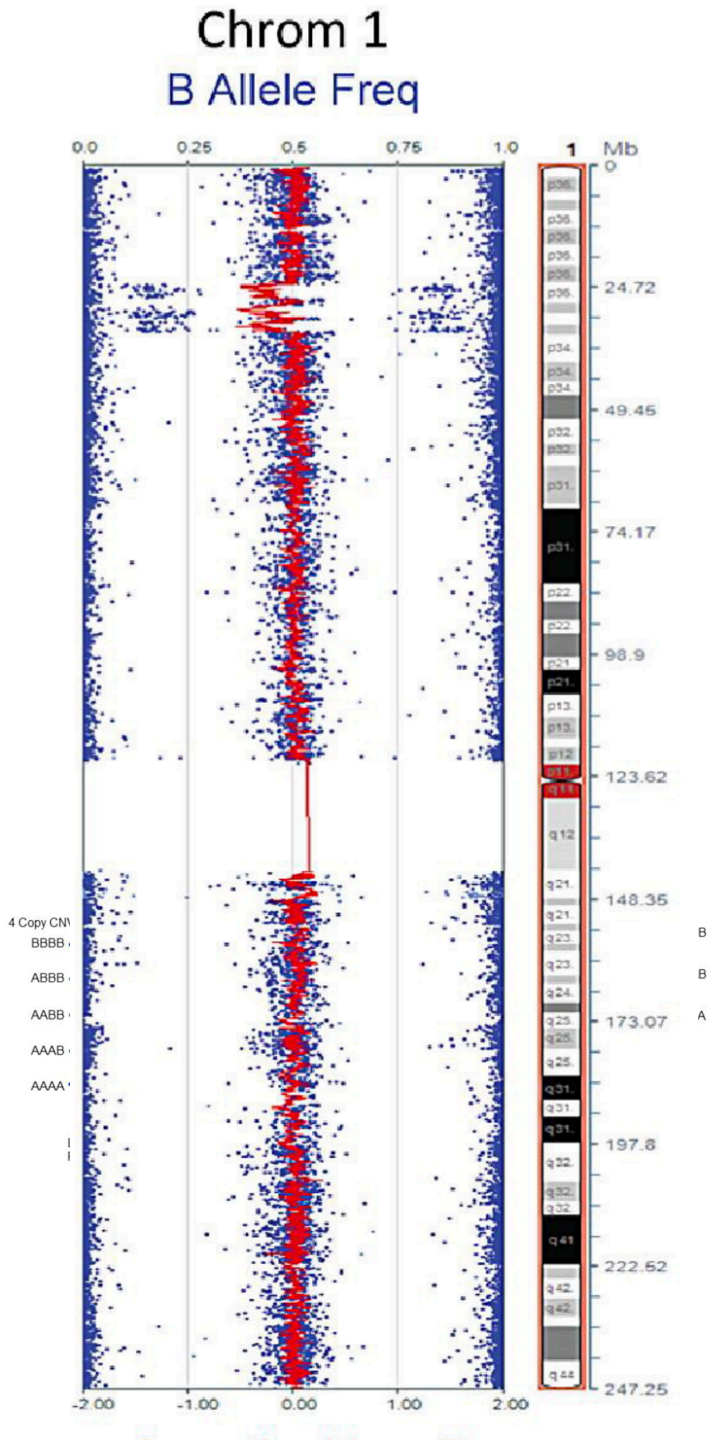
CMA分析的分辨率 100K以上



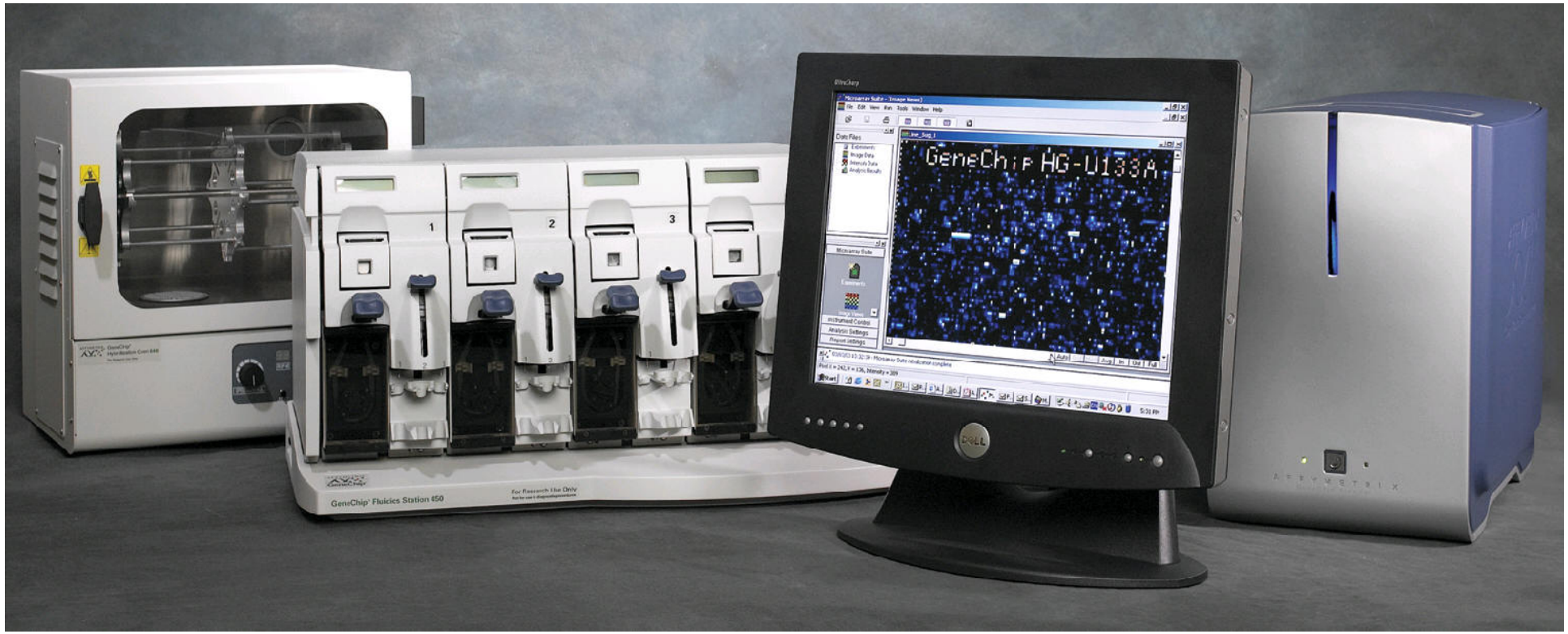




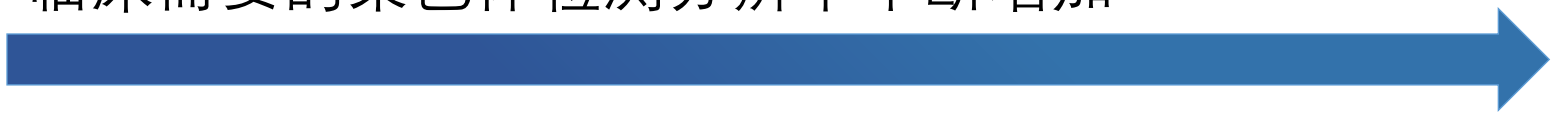




AffymetriX SNP-Microarray 系统



临床需要的染色体检测分辨率不断增加



- 倍体数异常
- 非整倍体异常
- 大片段结构异常

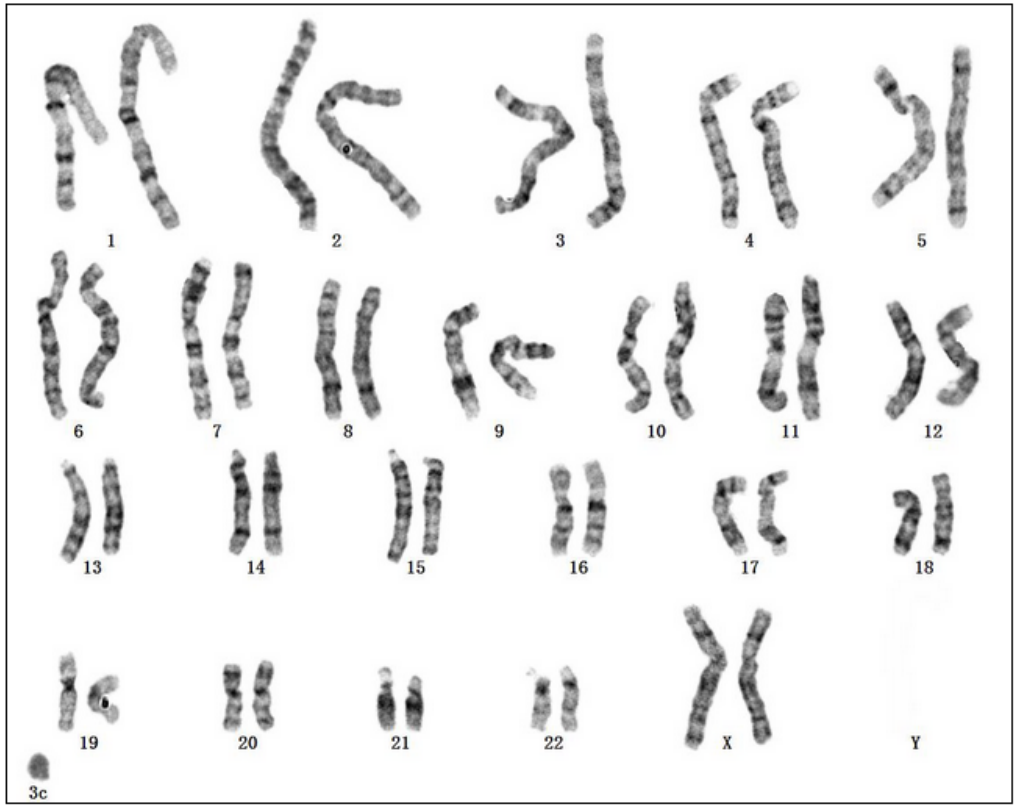
- 染色体微缺失
  微重复异常

- 基因缺失、突变

Microarray可以帮助核型分析提高诊断的精准性

王*， 28岁，产前筛查21三体高危1/70，行羊水穿刺提示47，XX+mar[4]/46,XX[46]

已排除了夫妇双方的染色体异常（核型分析均正常）

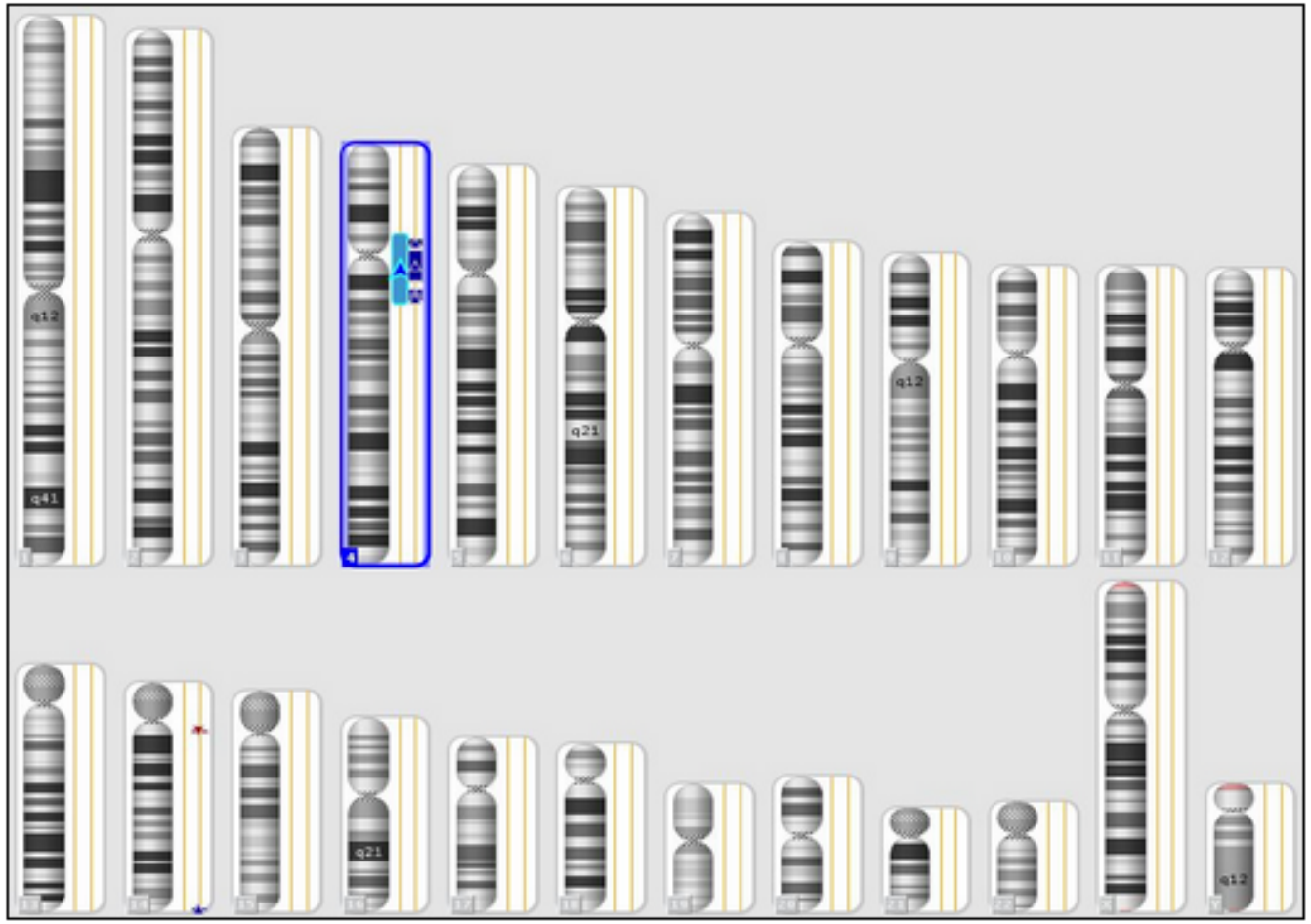


- Marker染色体指异常衍生的染色体，往往来源不明
- 有时带有重复的染色体片段，有时不含有关键性的基因
- 有时在正常人群中发现而没有表型
- 临床意义不明
- 需要明确衍生染色体的来源，含有的片段具体位置才能准确解析
- 该病例中由于没有目标性的判断，难以从FISH检测中找到原因

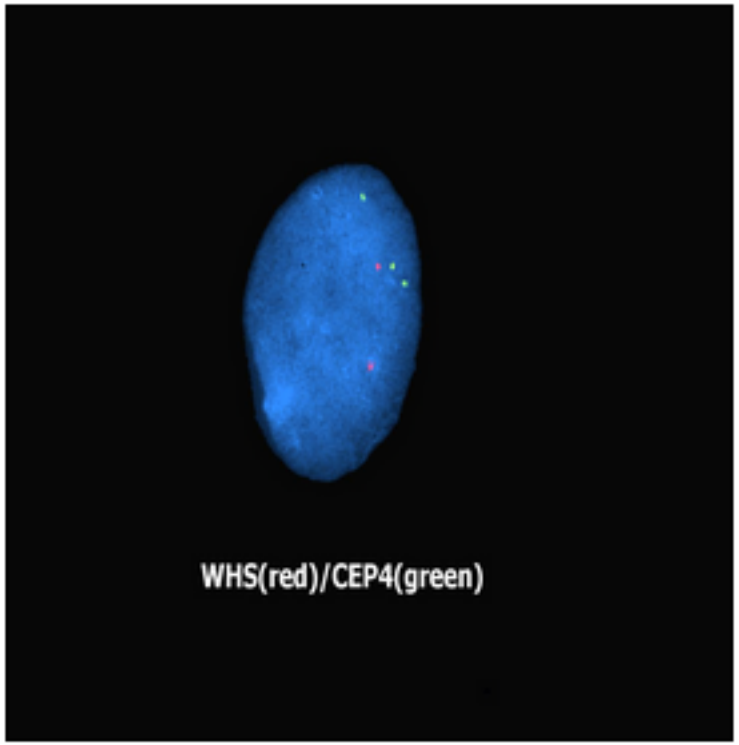
Microarray 是目前可以选择的好方法

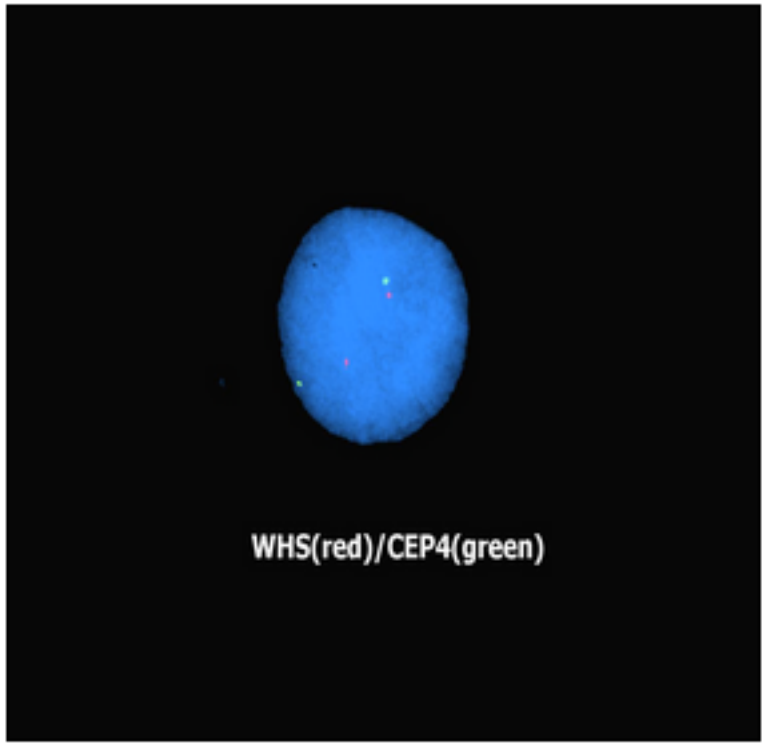
因无法从核型上辨认marker染色体的来源，于是再次穿刺抽取羊水行Microarray检测

Microarray检测显示胎儿4 号染色体4p13q13.3区段有30.537Mb的嵌合性重复



根据Microarray的提示，选择4号染色体的着丝粒探针进行FISH验证，得到确认28%的间期细胞提示有4号染色体着丝粒三体，说明了marker染色体的来源





- Microarray检测患者在4 号染色体4p13q13.3区段有30.537Mb的嵌合性重复
- 该片段包含有着丝粒，4p13,4 和 q13.3 部分
- 芯片检测显示65% 的细胞含有此重复片段
- 此片段含有SLC30A9, ATP8A1, GRXCR1, GNPDA2, GABRG1, GABRA2, COX7B2, GABRA4, GABRB1, CORIN, CNGA1, TXK 等82个OMIM基因。

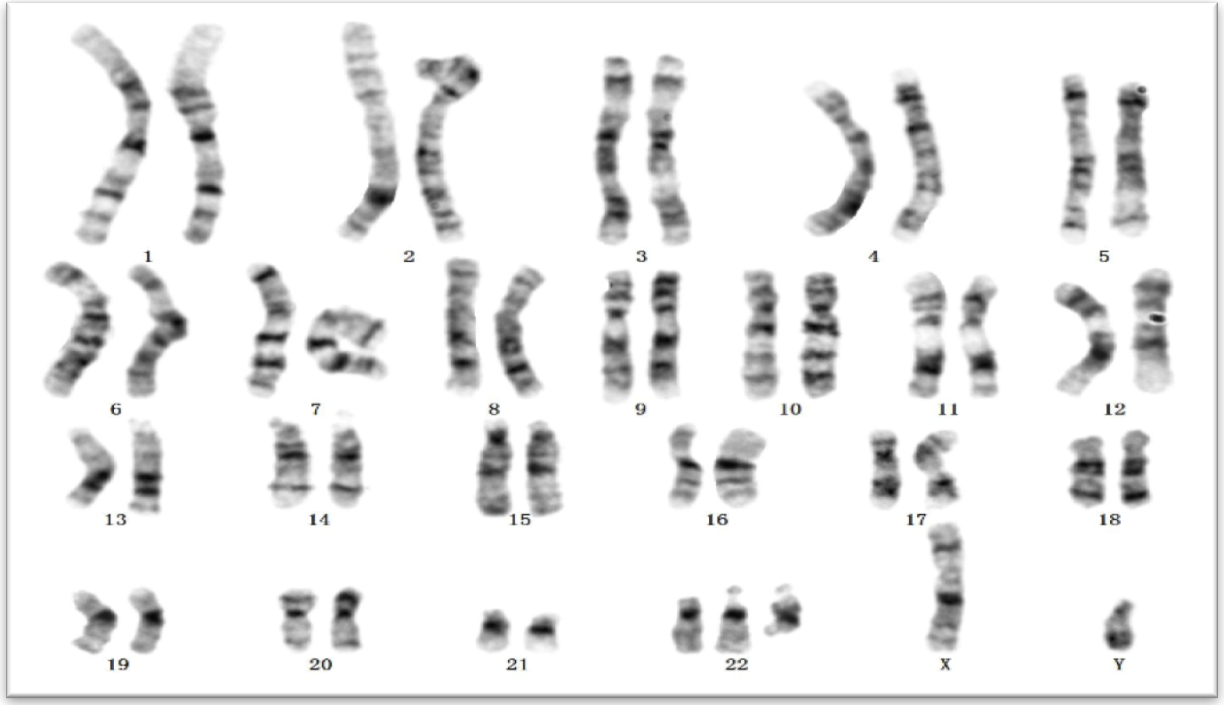
明确诊断后根据Microarray的检测结果进行详细的数据库检索和病例检索：

- 有报告4p12 - 4p11 重复的患者有多发性肾囊肿，皮肤肿瘤，癫痫发作
- 4p13 - 4p11 重复的患者有自闭症，智力发育迟缓
- 4p14 - 4q13.1 重复的患者有消化道异常，惊厥，智力残疾，运动迟缓
- 4q11 - 4q12重复的患者有遗传性遗忘失用症, 延迟的语言的进展,智力残疾
- 4q13.1 - 4q13.2 重复的患者有异常的刻板行为
- 本患者重复片段远大于以上患者的小部分重复

- 经过产前咨询，告知核型及FISH、Microarray的检测结果及分析报告
- 最终夫妇双方选择了终止妊娠

病例：microarray能帮助我们迅速找到染色体重复区域的定位

- 贾XX，32岁，G1P0
- 孕12周NT测定1.2mm
- 中孕期唐氏筛查低危
- 孕22周胎儿系统超声提示双侧脑室增宽1.2mm，双肾回声增强
- NIPT提示22号染色体“有重复”
- 23周我院行羊水穿刺，送检核型分析及microarray

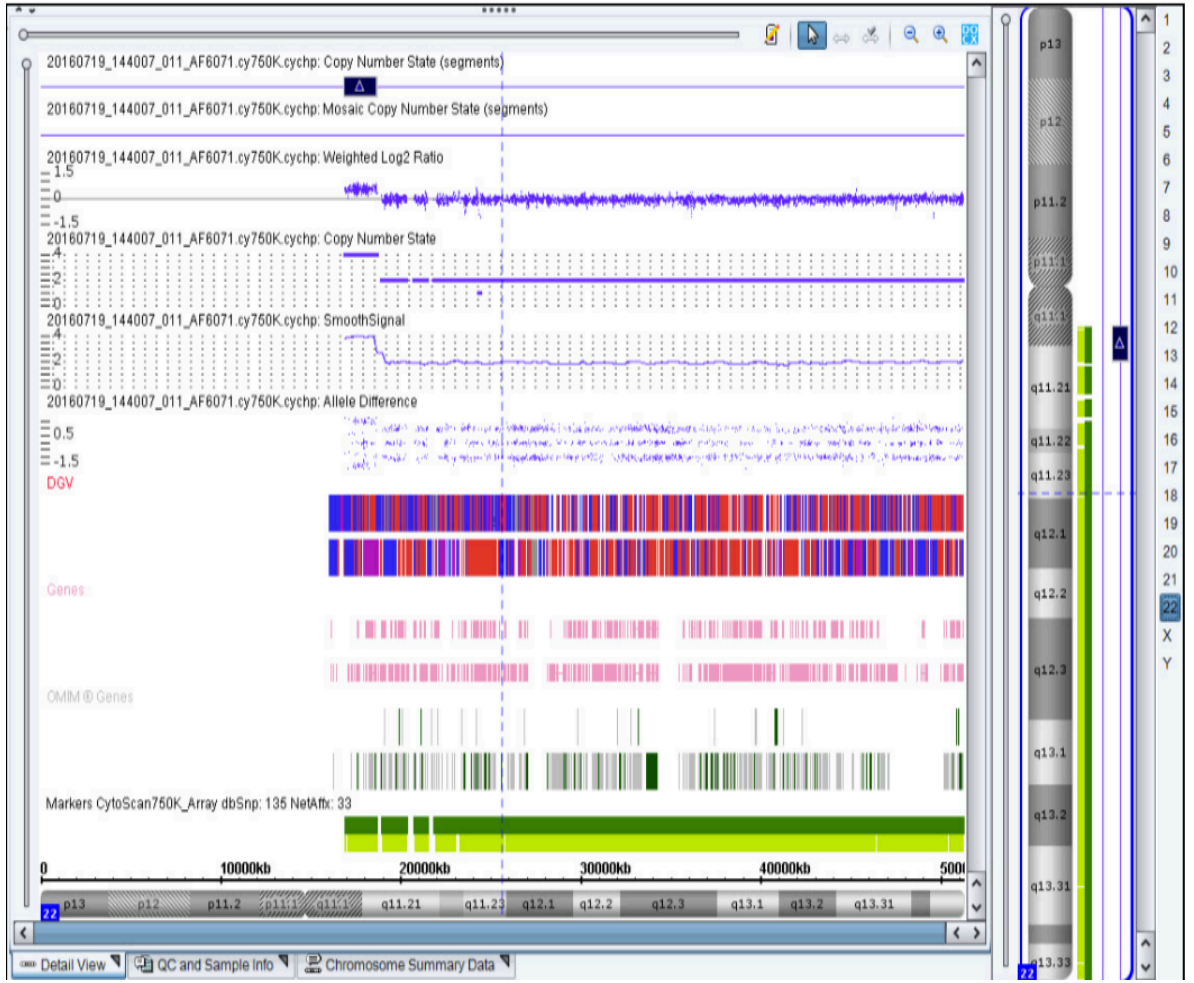


47，XY，+mar，del（22）？

病例-microarray结果

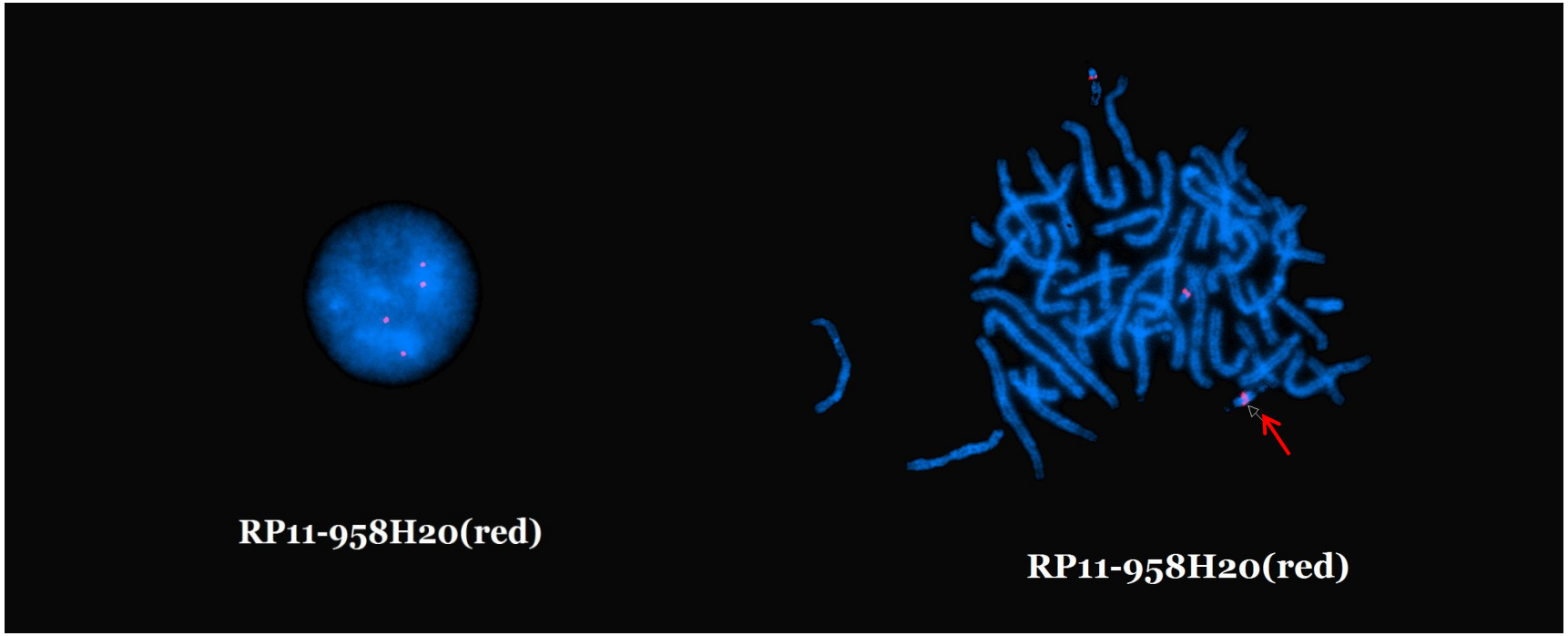
arr[hg19] Yq11.23(26,527,669-27,456,495)x0,
22q11.1q11.21(16,888,899-18,649,190)x4

- Microarray芯片检测结果显示胎儿Y染色体Yq11.23区段存在928.8Kb片段的缺失。位于AZFc区域,内含DAZ2,DAZ3等3个OMIM基因,包含了sY587和部分sY255位点. 含有DAZ1/DAZ2,DAZ3/DAZ4的Y染色体长臂AZFc区段的缺失是男性无/寡精,不育的原因之一。
- 胎儿22号染色体22q11.1q11.21区段存在1.76Mb的2次重复, 内含XKR, CECR1, CECR2, ATP6V1E1,MICAL,PEX2,TUBA8等 11个OMIM基因，涉及Cat eye syndrome (CES) 猫眼综合征的关键区域。CECR1,CECR2 和ATPV1E1 等OMIM基因是CES 猫眼综合征的候选基因.
- 夫妇双方的染色体核型分析及microarray是正常的



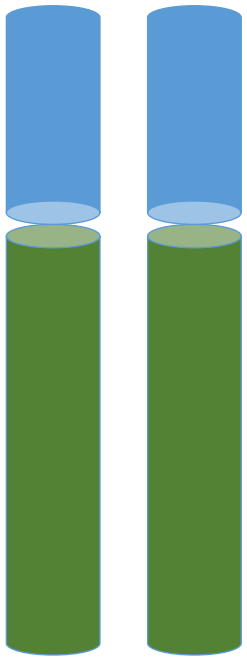
同时定制了22q11.1-22q11.2区段的BAC探针进行中期FISH的验证

FISH结果：47,XN,+mar.ish idic(22)(q11.2)(RP11-958H20++)

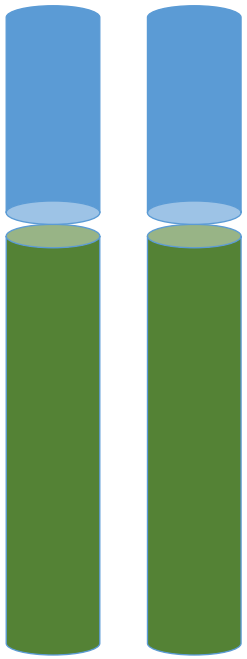


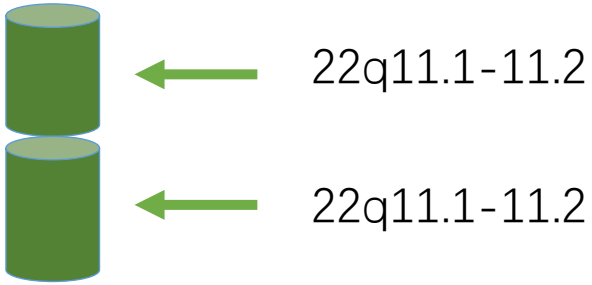
结果解释：
位于22q11.1-22q11.2区段内的BAC探针RP11-958H20 的FISH,结合G-带染色体分析和SNP芯片检测结果可以确认胎儿的额外标记染色体为来自22号染色体含有双份此探针位点片段的idic(22)(q11.2)衍生染色体。

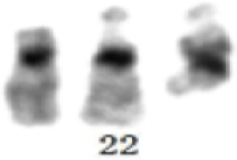
胎儿发生了什么？



正常人的22号染色体







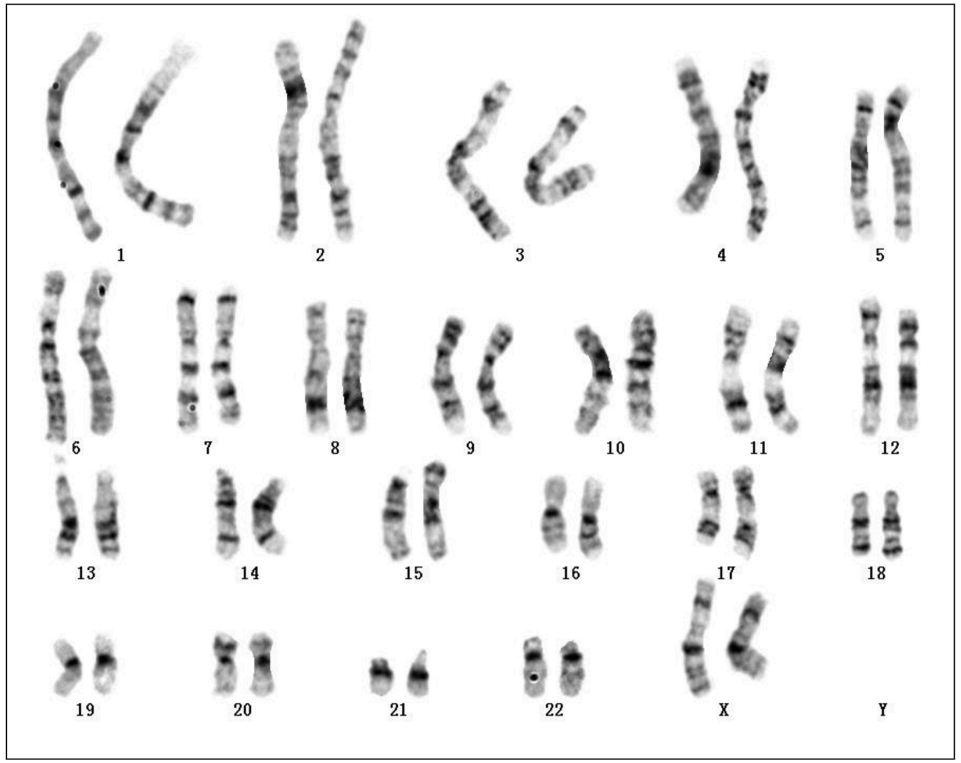
该胎儿的22号染色体组成

病例：最终诊断

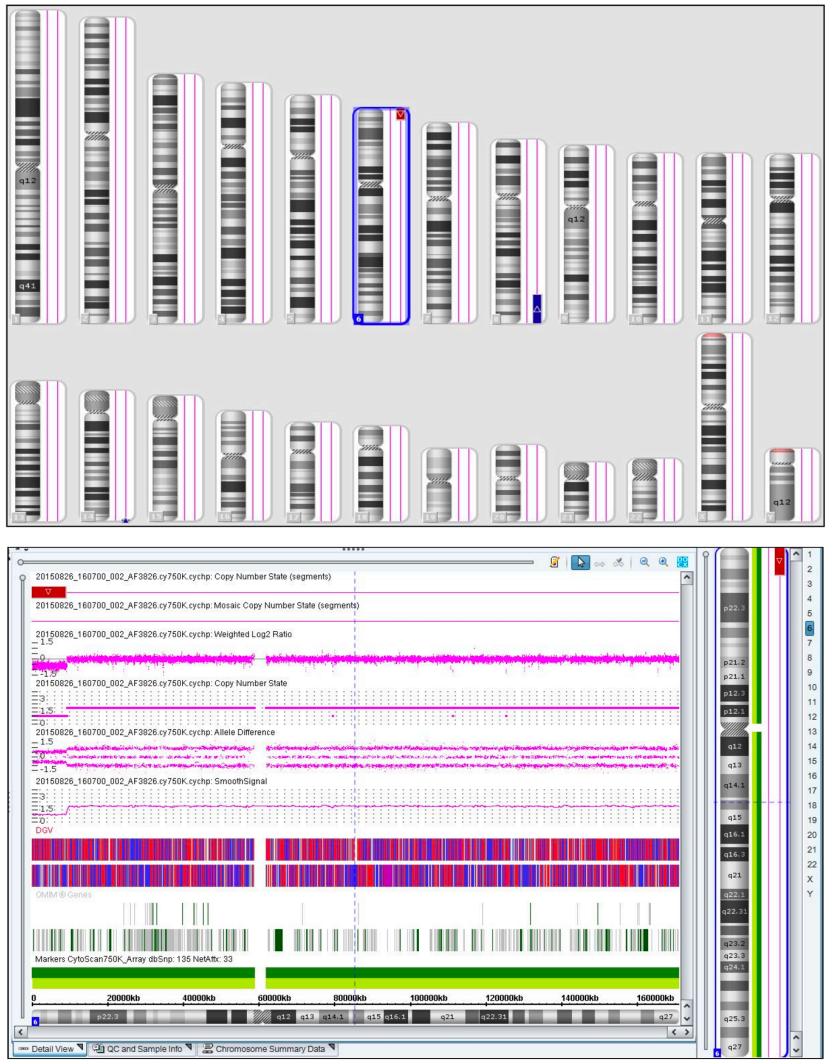
- 胎儿诊断结果：47,XN,idic(22)(q11.2),+22
- 胎儿含有一条额外的由两条22号染色体断裂点位于22q11.2对接而形成的含有双份22号短臂，着丝粒，部分长臂的异常双着丝粒等臂染色体，为猫眼综合症患者
- 绝大多数CES猫眼综合征的患者含有一条额外的包含2份以上候选基因位点片段的22 号染色体部分长臂的idic(22)(q11.2)标记染色体而导致以上基因片段的4倍体性
- 其临床特征是虹膜缺损伴有肛门闭锁/瘘；其他临床异常表现随患者的额外的22号染色体长臂等臂标记染色体所包含重复片段的大小而有不同程度的异常, 包括智力发育低下，胎儿生长发育迟缓, 先天的下斜睑裂, 眼, 耳, 鼻等面部, 头部异常和心脏,肾脏发育畸形等异常

Microarray可以帮助核型不明确的病例诊断

28岁，G2P0，有一次自然流产，本次筛查高风险



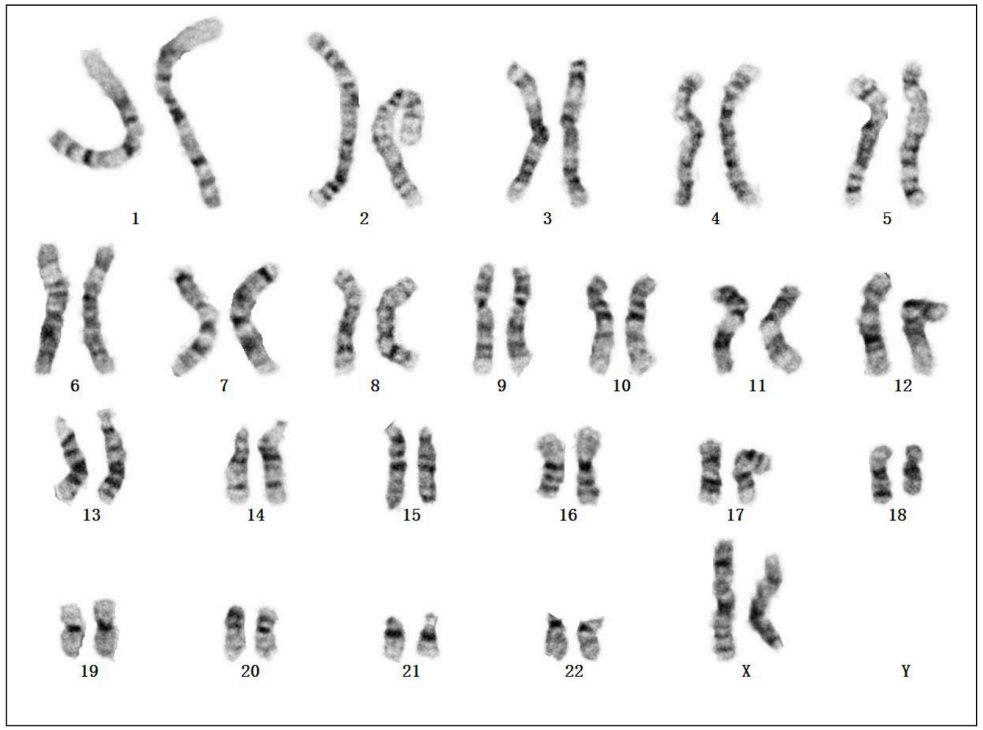
46, XN, der(6)t(6;?)(p23;?), add(15)(p11.2)



arr[hg19] 6p25.3p24.3(156,974-9,212,750)x1, 8q24.13q24.3(123,406,098-146,295,771)x3

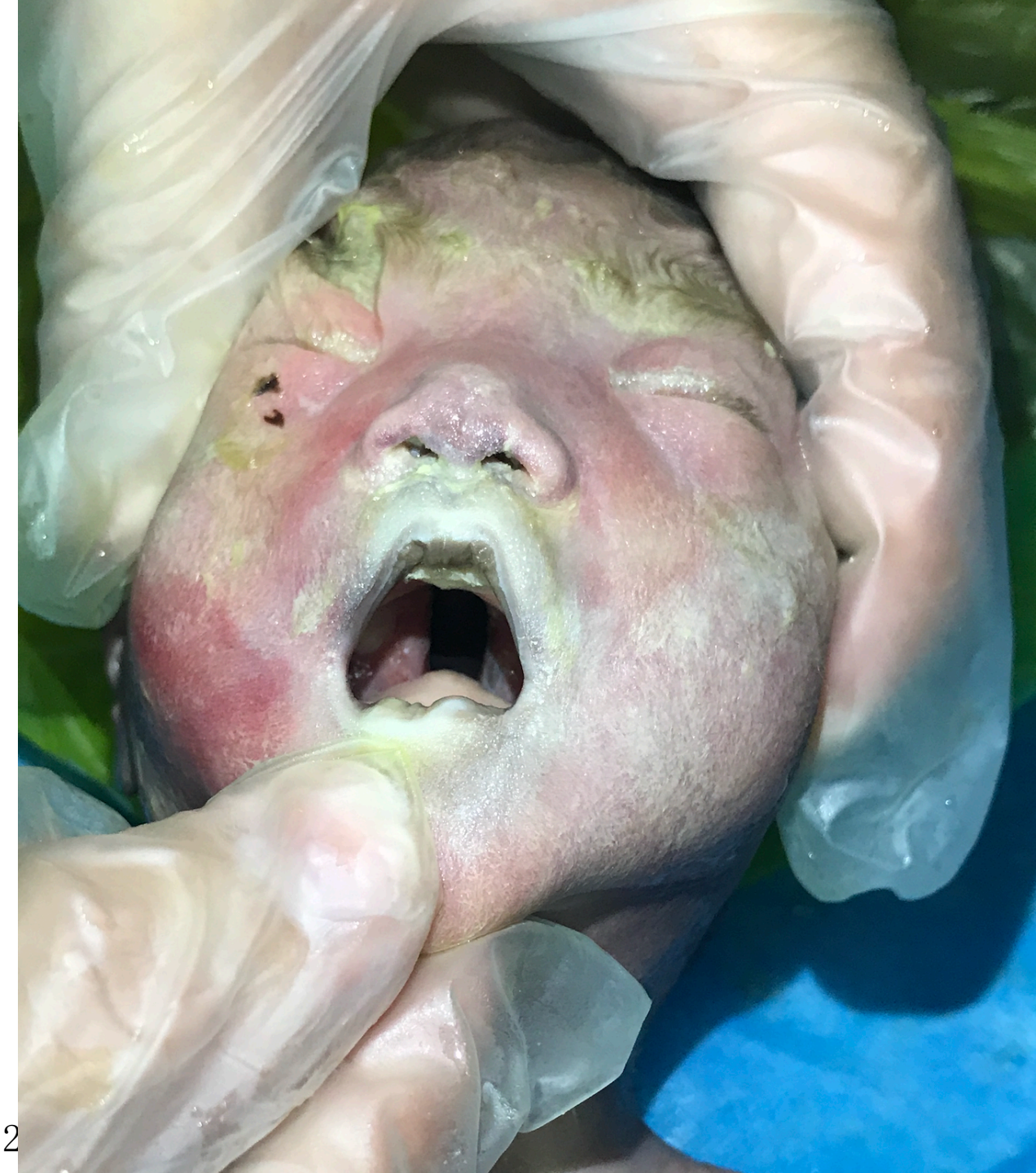
18号染色体片段缺失

33岁，G1P0，NF增厚，NIPT正常



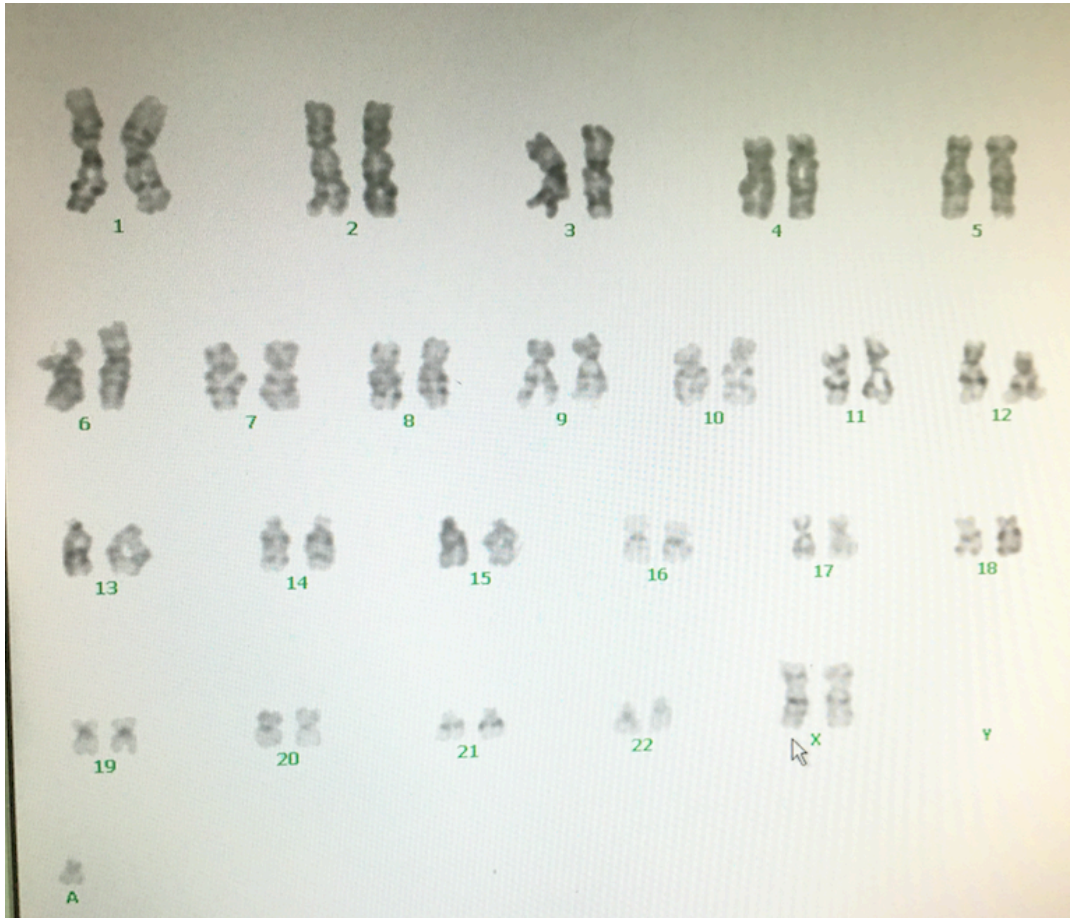
46,XX,del(18)(q21.3)

arr[hg19] 18p11.32p11.21(136,22



Microarray对于胎儿超声异常的遗传学诊断意义重大

- 马X，30岁，G1/P0
- 13周外院行NT筛查3.94mm
- 20周转至我院行羊水穿刺
- 等羊水穿刺结果期间23周系统超声提示左侧脑室后角增宽1.1cm
- 同期胎儿超声心动检查示先天性心脏病（室缺，主动脉弓发育不良，离断型）
- 实验室取羊水上清液送检microarray

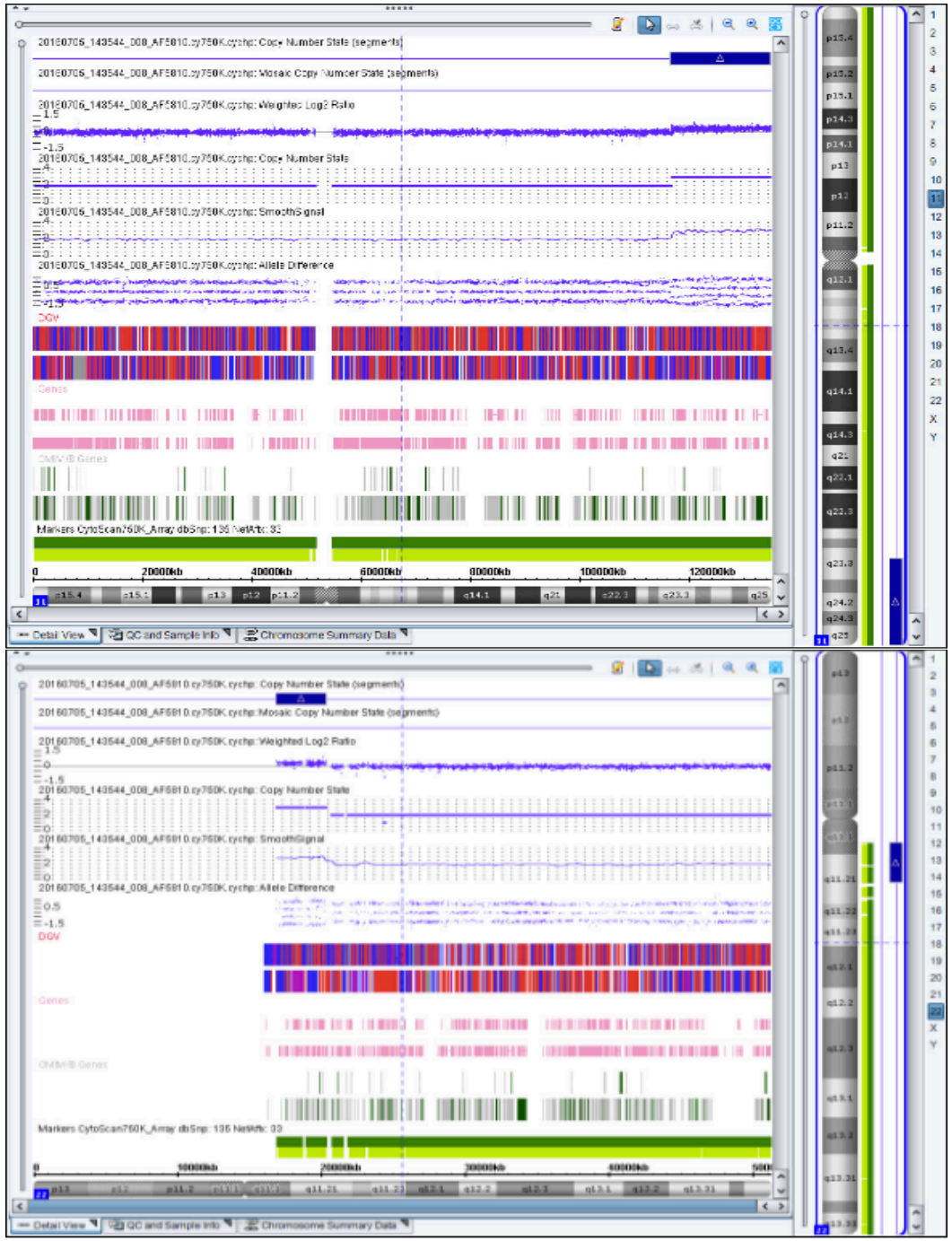


47，XN，+mar

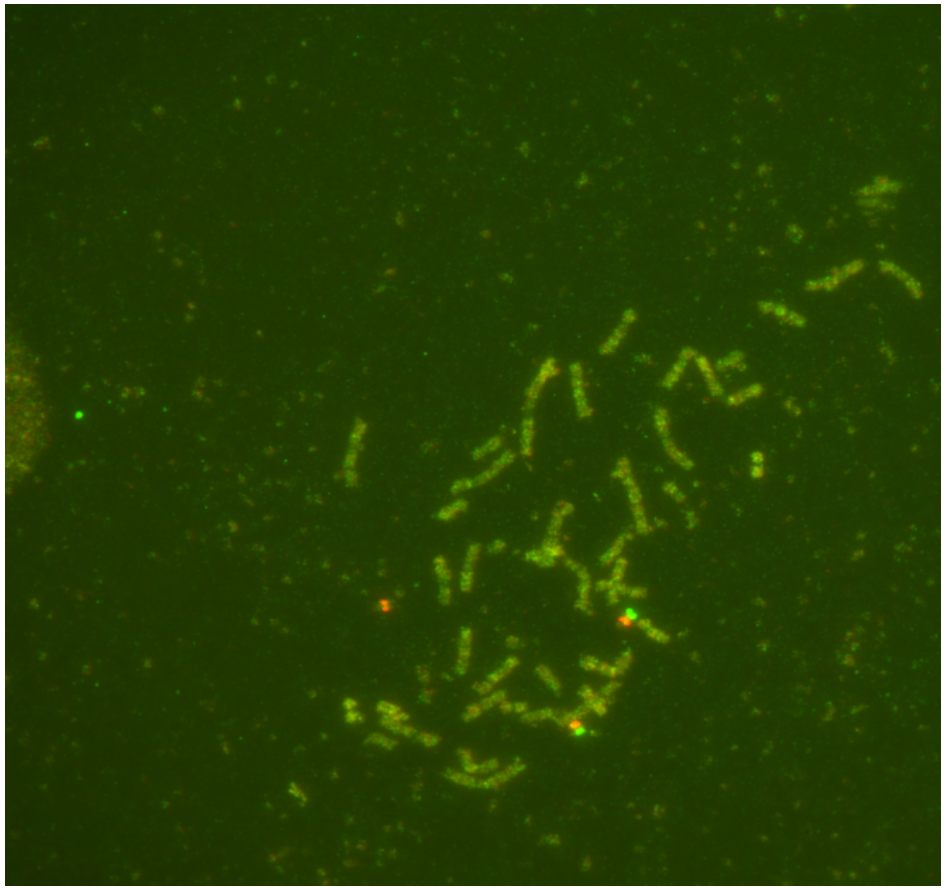
病例：microarray结果

arr[hg19] 11q23.3q25(116,683,754-134,937,416)x3,
22q11.1q11.21(16,888,899-20,312,661)x3

- 11号染色体11q23.3q25区段存在18.2Mb片段的重复，内含KCNJ1，FLI1，JAM3等123个OMIM基因，涉及Jacobsen综合征疾病区域。已有研究报道该片段重复与头面部异常，智力低下，语言发育迟缓等疾病相关。
- 22号染色体22q11.1q11.21区段存在3.4Mb片段重复，内含CLTCL1，HIRA，TBX1等37个OMIM基因，涉及Emanuel综合征（Emanuel syndrome）、22q11.2微重复综合征（Chromosome 22q11.2 microduplication syndrome）和22q11重复综合征(22q11 duplication syndrome)疾病区域
- 临床表型包括小头畸形，小颌畸形，心脏缺损，智力低下，精神运动发育延迟，生长迟滞，肌张力减退等

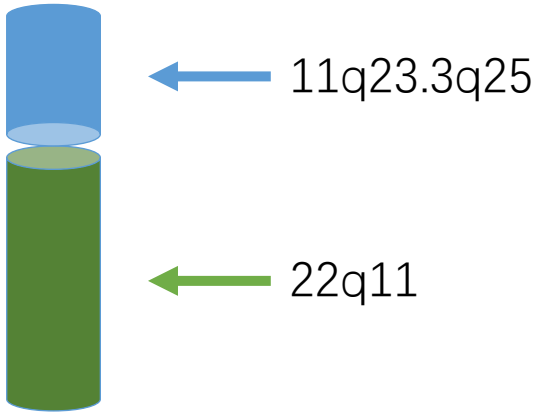


病例：结合FISH诊断结果



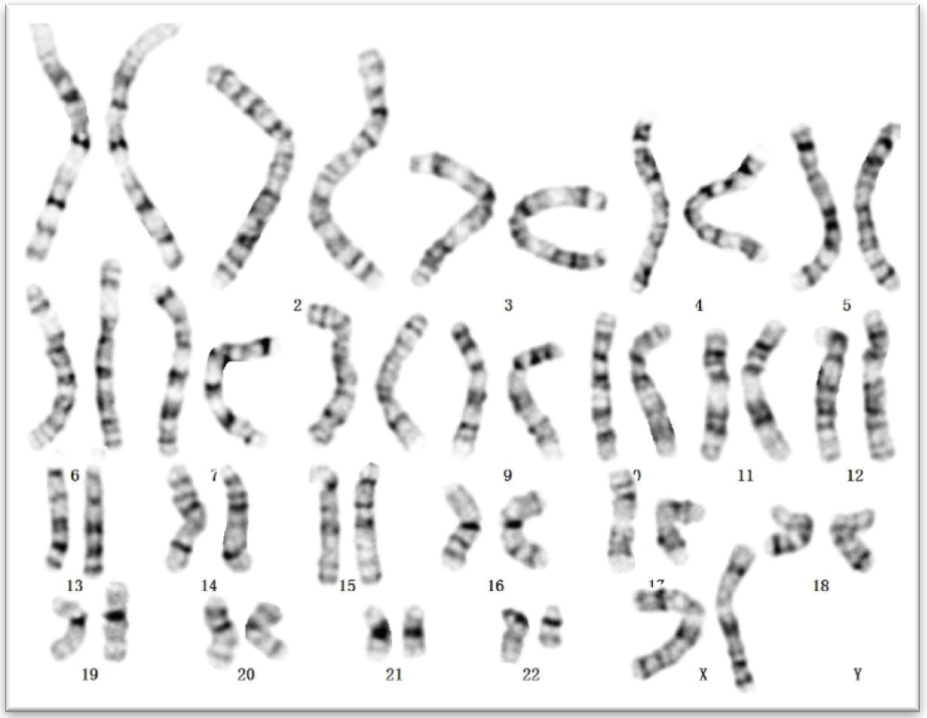
GLP TUPLE1（22q11）红 /GLP ARSA（22q13）绿

FISH检测说明了该衍生染色体是由
11q23.3q25和22q11的染色体片段组合而成

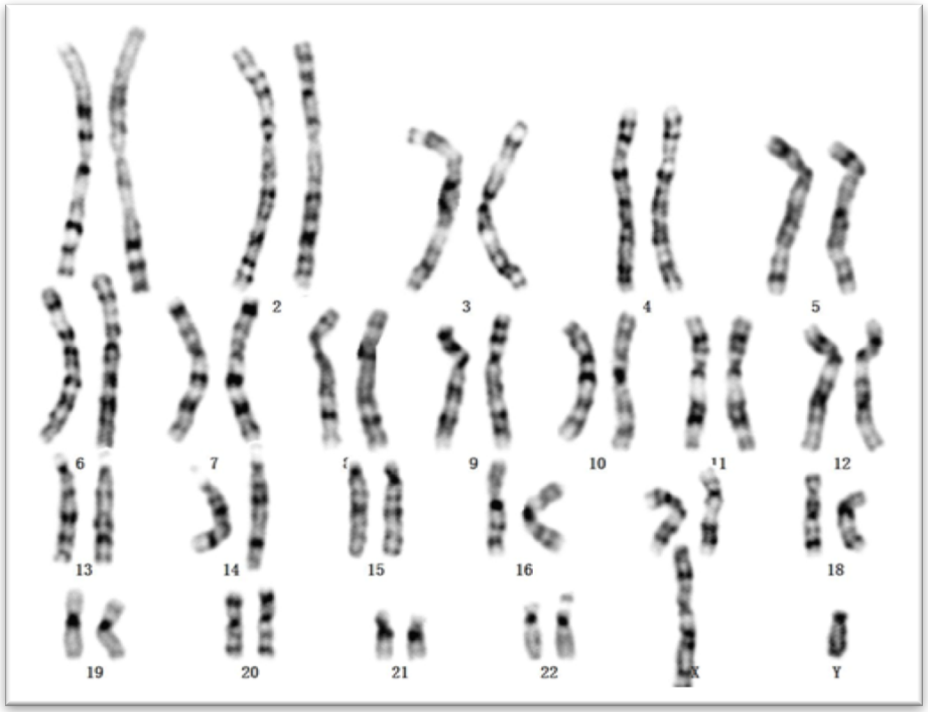


Marker 染色体

下一胎还会再出现吗？再发风险的评估-夫妇外周血染色体分析

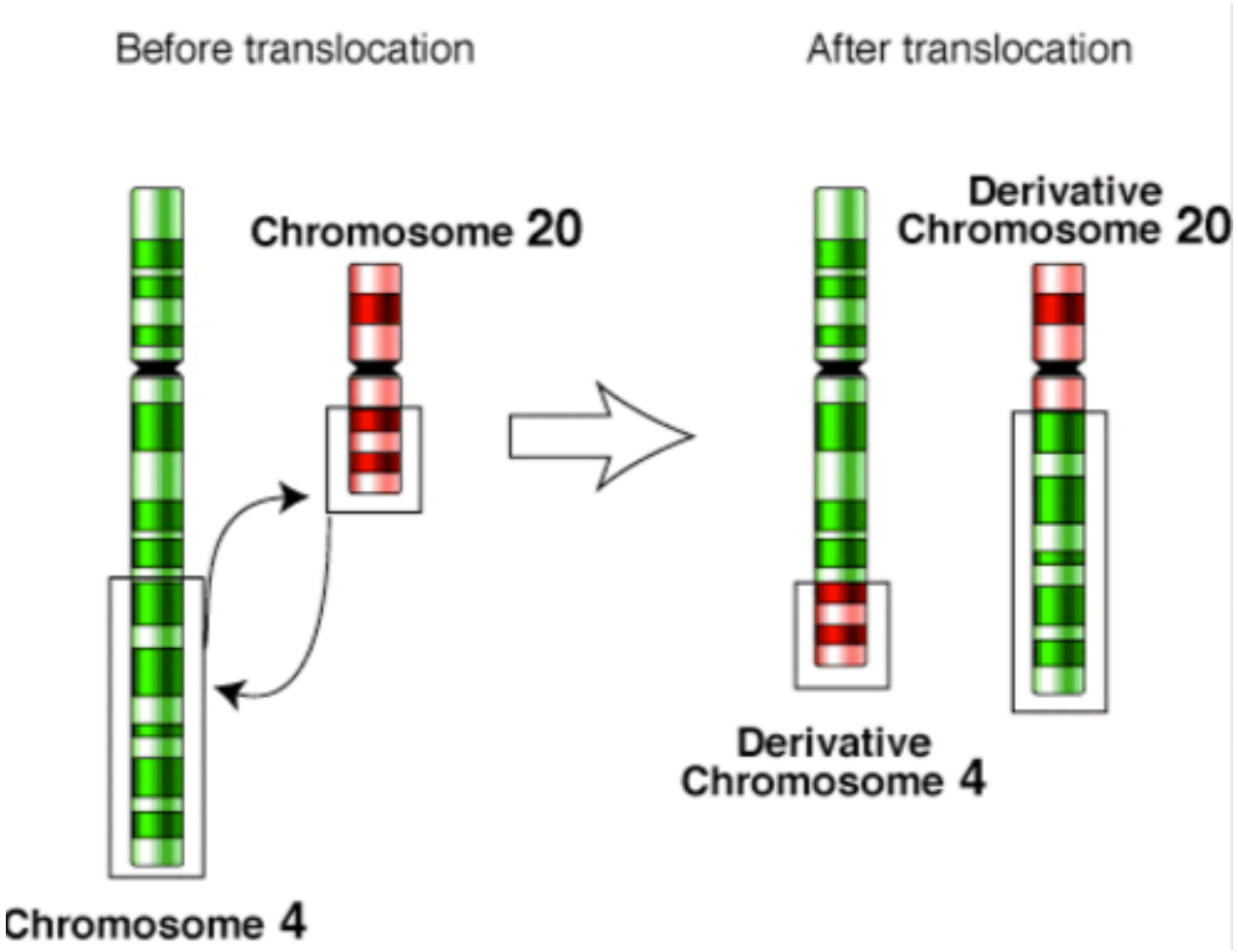


46,XX,t(11;22)(q23.3;q11.2)

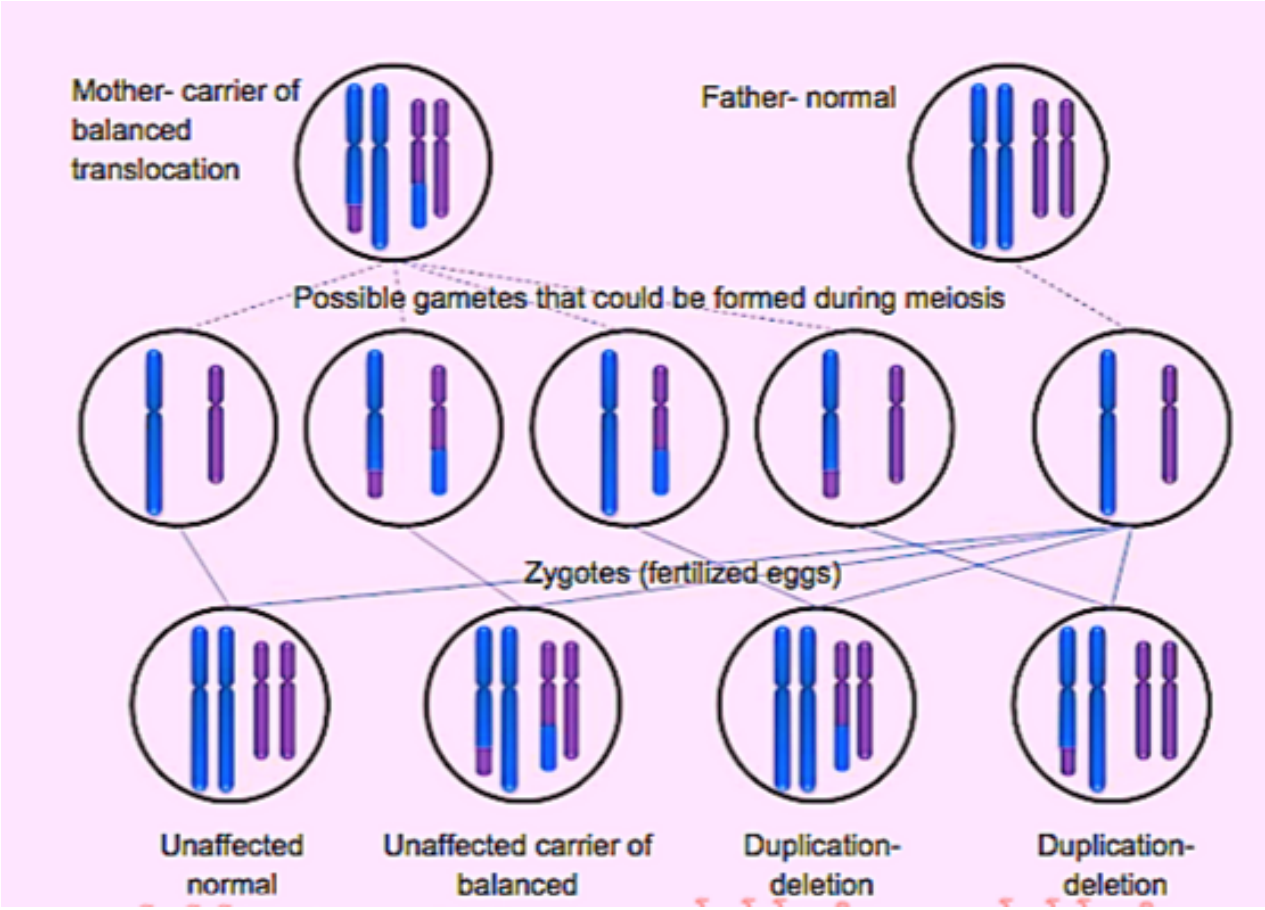


46,XY

染色体平衡易位的发生

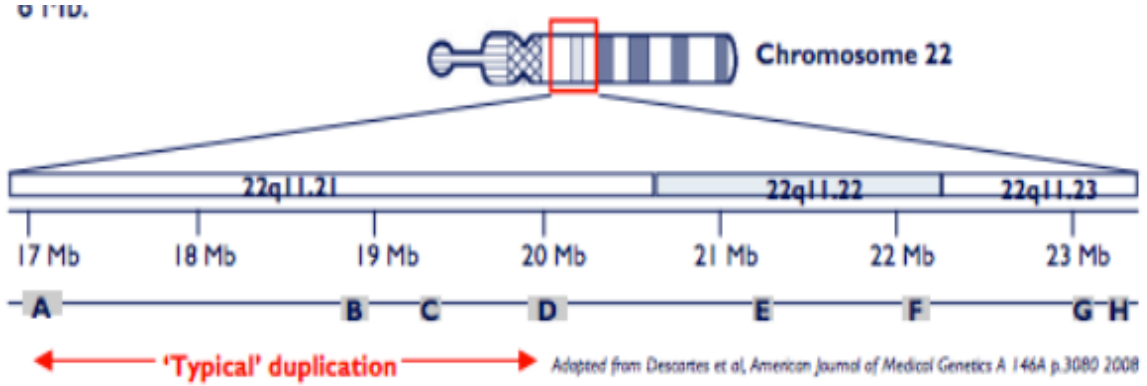

Before translocation
After translocation
Chromosome 20
Derivative Chromosome 20
Derivative Chromosome 4
Chromosome 4

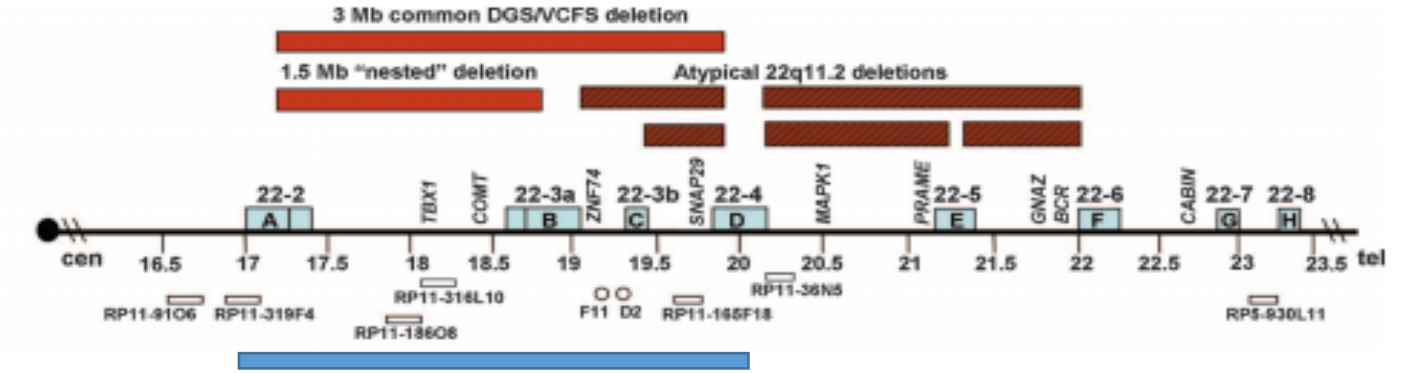
平衡易位的患者后代易出现反复的流产



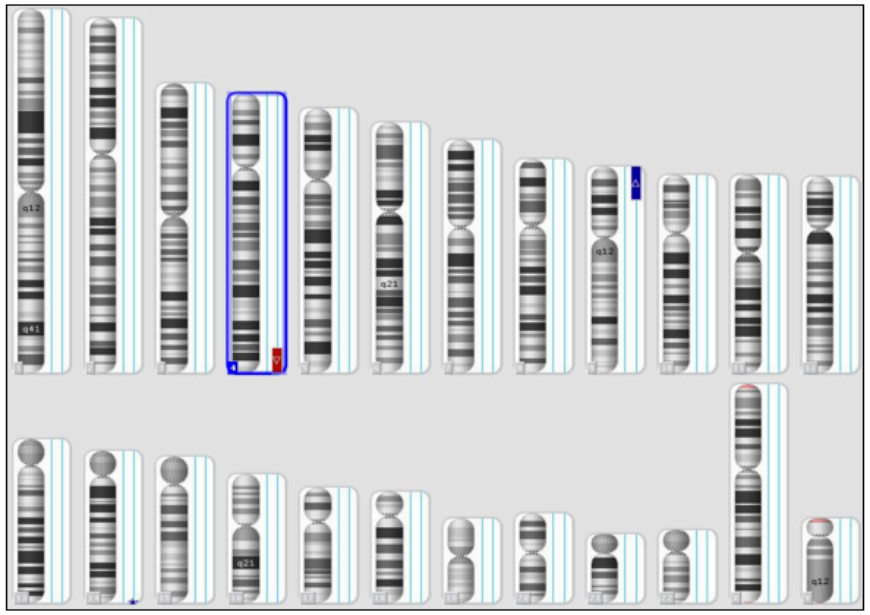
22q11.2微重复综合征

- 多种先天性心脏病
- 颚咽综合征
- 听力损害
- 体格发育迟滞
- 学习认知能力下降 .....



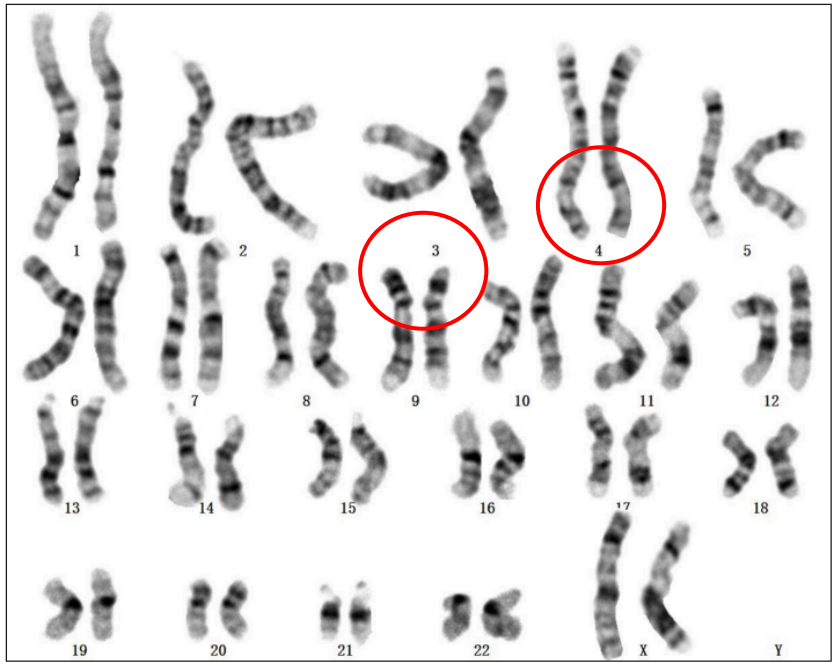


类似的病例：胎儿先天性心脏病，宫内发育迟缓行羊水穿刺诊断

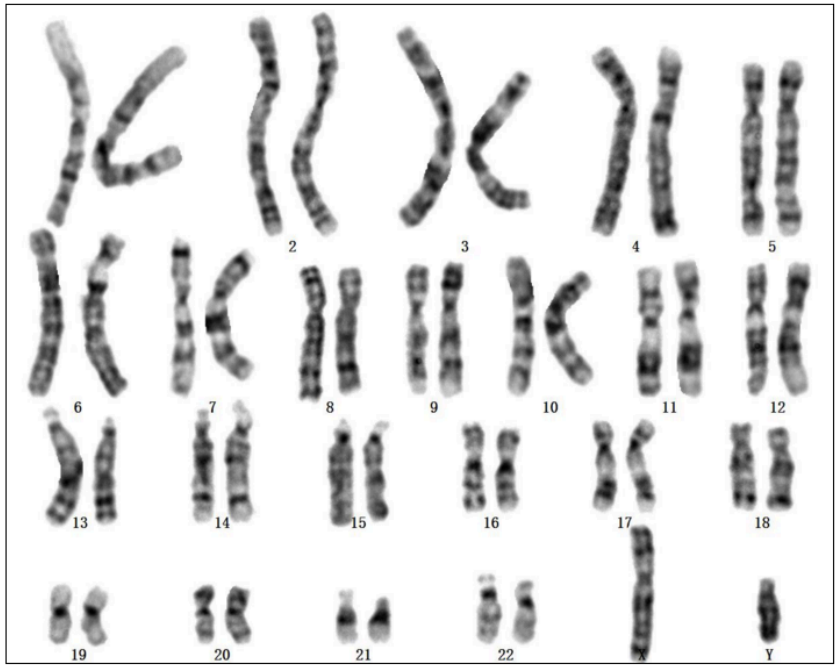


- 4号染色体4q34.1q35.2存在16.531Mb片段的缺失，含有SPATA4，VEGFC，NEIL3 等73个OMIM基因，该片段的缺失与大头畸形，小颌畸形等面部异常，肺部异常，心脏疾病，全身发育迟缓等表型有关
- 9号染色体9p24.3p21.3存在22.342Mb片段的重复，含有 VLDLR，KCNV2，RFX3 等102个OMIM基因，该片段的重复与智力障碍，语言发育迟缓，脑部发育异常等表型有关

亲代的染色体核型分析



46,XX,t(4;9)(q34;p21)



46,XY

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Chromosomal Microarray versus Karyotyping for Prenatal Diagnosis

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ABSTRACT

BACKGROUND

Chromosomal microarray analysis has emerged as a primary diagnostic tool for the evaluation of developmental delay and structural malformations in children. We aimed to evaluate the accuracy, efficacy, and incremental yield of chromosomal microarray analysis as compared with karyotyping for routine prenatal diagnosis.

METHODS

Samples from women undergoing prenatal diagnosis at 29 centers were sent to a central karyotyping laboratory. Each sample was split in two; standard karyotyping was performed on one portion and the other was sent to one of four laboratories for chromosomal microarray.

RESULTS

We enrolled a total of 4406 women. Indications for prenatal diagnosis were advanced maternal age (46.6%), abnormal result on Down's syndrome screening (18.8%), structural anomalies on ultrasonography (25.2%), and other indications (9.4%). In 4340 (98.8%) of the fetal samples, microarray analysis was successful; 87.9% of samples could be used without tissue culture. Microarray analysis of the 4282 nonmosaic samples identified all the aneuploidies and unbalanced rearrangements identified on karyotyping but did not identify balanced translocations and fetal triploidy. In samples with a normal karyotype, microarray analysis revealed clinically relevant deletions or duplications in 6.0% with a structural anomaly and in 1.7% of those whose indications were advanced maternal age or positive screening results.

CONCLUSIONS

In the context of prenatal diagnostic testing, chromosomal microarray analysis identified additional, clinically significant cytogenetic information as compared with karyotyping and was equally efficacious in identifying aneuploidies and unbalanced rearrangements but did not identify balanced translocations and triploidies. (Funded by the Eunice Kennedy Shriver National Institute of Child Health and Human Development and others; ClinicalTrials.gov number, NCT01279733.)

From the Departments of Obstetrics and Gynecology (R.J.W., M.S.) and Pathology and Cell Biology (B.L., V.S.A., O.N.), Columbia University Medical Center, Carnegie Hill Imaging for Women (D.S.), and Montefiore Medical Center/Albert Einstein College of Medicine (S.K.) — all in New York; the Department of Human Genetics, Emory University School of Medicine, Atlanta (C.L.M., B.B., D.H.L.); Signature Genomic Laboratories, Spokane, WA (B.C.B., A.N.L., L.G.S.); the Department of Molecular and Human Genetics, Baylor College of Medicine, Houston (C.M.E., A.P., A.L.B.); George Washington University Biostatistics Center, Rockville, MD (J.M.Z., E.A.T.); Center for Fetal Medicine and Women's Ultrasound, Los Angeles (L.D.P.); Feinberg School of Medicine, Northwestern University, Chicago (W.A.G.); Integrated Genetics, Westborough, MA (T.S.), and Santa Fe, NM (K.M.); Florida International University, Miami (J.L.S.); and Drexel University College of Medicine, Philadelphia (L.J.). Address reprint requests to Dr. Wapner at Columbia University Medical Center, Department of Obstetrics and Gynecology, 622 W. 168th St, PH16-66, New York, NY 10032, or at rw2191@mail.cumc.columbia.edu.

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Microarray是诊断胎儿超声异常背后的染色体病的首要技术

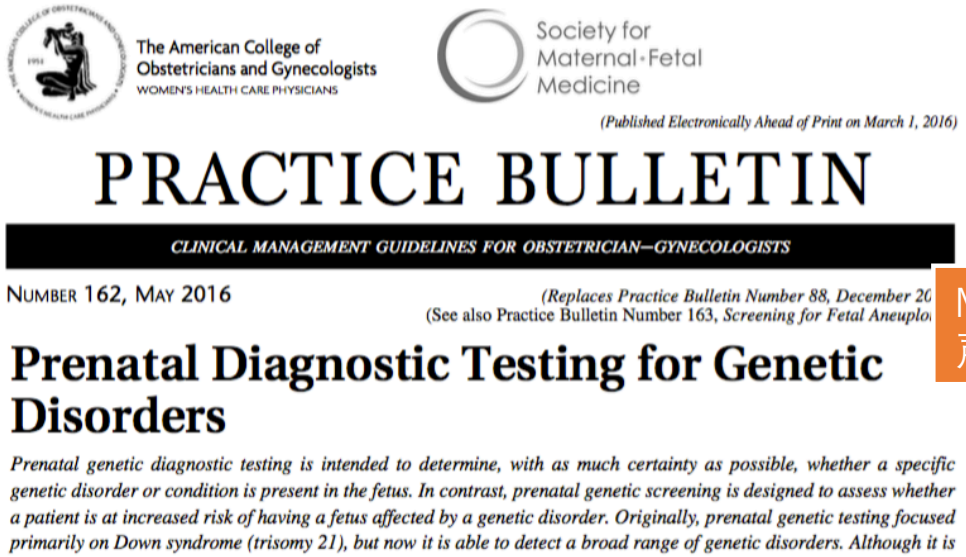
Table 3. Frequency and Clinical Interpretation of Microdeletions and Duplications on Chromosomal Microarray in the 3822 Samples with a Normal Karyotype, According to Indication for Prenatal Testing.

Indication for Prenatal Diagnosis	Normal Karyotype	Common Benign	Pathogenic	Uncertain Clinical Significance (N=130)		Total Known Pathogenic and Potential for Clinical Significance*
				Likely to Be Benign	Potential for Clinical Significance	
	no.		*no. (%)*			*no. (%) [95% CI]†*
Any	3822	1234 (32.3)	35 (0.9)	69 (1.8)‡	61 (1.6)	96 (2.5) [2.1–3.1]
Advanced maternal age	1966	628 (31.9)	9 (0.5)	37 (1.9)	25 (1.3)	34 (1.7) [1.2–2.4]
Positive on Down's syndrome screening	729	247 (33.9)	3 (0.4)	13 (1.8)	9 (1.2)	12 (1.6) [0.9–2.9]
Anomaly on ultrasonography	755	247 (32.7)	21 (2.8)	16 (2.1)	24 (3.2)	45 (6.0) [4.5–7.9]
Other§	372	112 (30.1)	2 (0.5)	3 (0.8)	3 (0.8)	5 (1.3) [0.6–3.1]

2016年美国妇产科学会指南

核型分析或microarray可以适用于任何一例产前诊断的病例

Microarray检测应该用于所有胎儿超声有异常结果的产前诊断病例



The American College of Obstetricians and Gynecologists
WOMEN'S HEALTH CARE PHYSICIANS

Society for Maternal-Fetal Medicine

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PRACTICE BULLETIN

CLINICAL MANAGEMENT GUIDELINES FOR OBSTETRICIAN–GYNECOLOGISTS

NUMBER 162, MAY 2016

(Replaces Practice Bulletin Number 88, December 20
(See also Practice Bulletin Number 163, *Screening for Fetal Aneuplo*

Prenatal Diagnostic Testing for Genetic Disorders

Prenatal genetic diagnostic testing is intended to determine, with as much certainty as possible, whether a specific genetic disorder or condition is present in the fetus. In contrast, prenatal genetic screening is designed to assess whether a patient is at increased risk of having a fetus affected by a genetic disorder. Originally, prenatal genetic testing focused primarily on Down syndrome (trisomy 21), but now it is able to detect a broad range of genetic disorders. Although it is

mutation that causes the disease. Karyotype or microarray analysis should be offered in every case, although performing karyotype or microarray may not be necessary in a low-risk patient. Also, routine measurement of amniotic fluid alpha fetoprotein to screen for neural tube defects may not be necessary in all cases when amniocentesis is performed for other indications and the ultrasound examination is normal with good visualization of the fetal spine and head (Table 1).

In patients with a major fetal structural abnormality found on ultrasound examination, CVS or amniocentesis with chromosomal microarray should be offered (10). If a structural abnormality is strongly suggestive of a particular aneuploidy in the fetus (eg, duodenal atresia or an atrioventricular heart defect, which are characteristic

thout FISH

ay analysis.

g offspring

with trisomy 13, 18, or 21 based on abnormal serum screening or cell-free DNA testing, amniocentesis with FISH plus karyotype or with karyotype alone should be offered. Additionally, chromosomal microarray analysis should be available to women undergoing invasive

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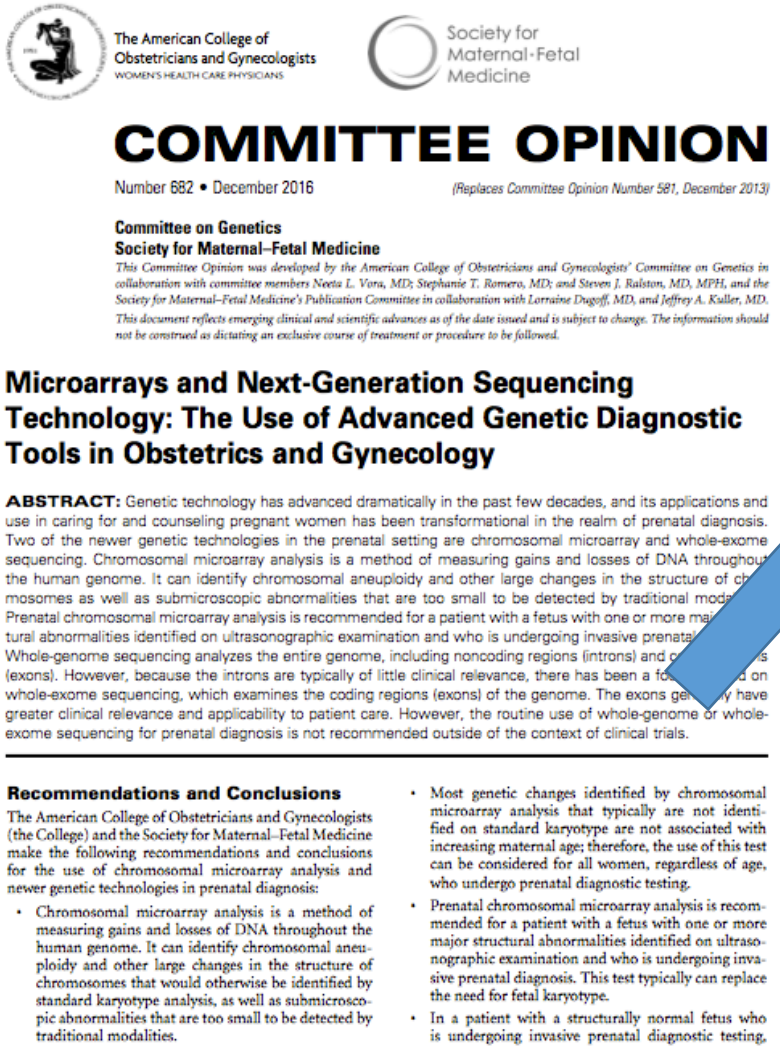
to a gene
training a



The American College of Obstetricians and Gynecologists
WOMEN'S HEALTH CARE PHYSICIANS

Society for Maternal-Fetal Medicine

COMMITTEE OPINION

Number 682 • December 2016 (Replaces Committee Opinion Number 581, December 2013)

Committee on Genetics
Society for Maternal–Fetal Medicine

This Committee Opinion was developed by the American College of Obstetricians and Gynecologists' Committee on Genetics in collaboration with committee members Neeta L. Vora, MD; Stephanie T. Romero, MD; and Steven J. Ralston, MD, MPH, and the Society for Maternal–Fetal Medicine's Publication Committee in collaboration with Lorraine Dugoff, MD, and Jeffrey A. Kuller, MD.

This document reflects emerging clinical and scientific advances as of the date issued and is subject to change. The information should not be construed as dictating an exclusive course of treatment or procedure to be followed.

Microarrays and Next-Generation Sequencing Technology: The Use of Advanced Genetic Diagnostic Tools in Obstetrics and Gynecology

ABSTRACT: Genetic technology has advanced dramatically in the past few decades, and its applications and use in caring for and counseling pregnant women has been transformational in the realm of prenatal diagnosis. Two of the newer genetic technologies in the prenatal setting are chromosomal microarray and whole-exome sequencing. Chromosomal microarray analysis is a method of measuring gains and losses of DNA throughout the human genome. It can identify chromosomal aneuploidy and other large changes in the structure of chromosomes as well as submicroscopic abnormalities that are too small to be detected by traditional modalities. Prenatal chromosomal microarray analysis is recommended for a patient with a fetus with one or more major structural abnormalities identified on ultrasonographic examination and who is undergoing invasive prenatal diagnosis. Whole-genome sequencing analyzes the entire genome, including noncoding regions (introns) and coding regions (exons). However, because the introns are typically of little clinical relevance, there has been a focus on whole-exome sequencing, which examines the coding regions (exons) of the genome. The exons generally have greater clinical relevance and applicability to patient care. However, the routine use of whole-genome or whole-exome sequencing for prenatal diagnosis is not recommended outside of the context of clinical trials.

Recommendations and Conclusions

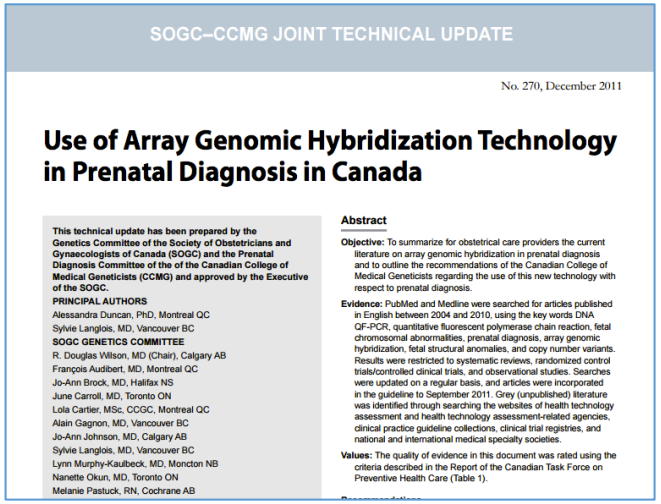
The American College of Obstetricians and Gynecologists (the College) and the Society for Maternal–Fetal Medicine make the following recommendations and conclusions for the use of chromosomal microarray analysis and newer genetic technologies in prenatal diagnosis:

- Chromosomal microarray analysis is a method of measuring gains and losses of DNA throughout the human genome. It can identify chromosomal aneuploidy and other large changes in the structure of chromosomes that would otherwise be identified by standard karyotype analysis, as well as submicroscopic abnormalities that are too small to be detected by traditional modalities.
- Most genetic changes identified by chromosomal microarray analysis that typically are not identified on standard karyotype are not associated with increasing maternal age; therefore, the use of this test can be considered for all women, regardless of age, who undergo prenatal diagnostic testing.
- Prenatal chromosomal microarray analysis is recommended for a patient with a fetus with one or more major structural abnormalities identified on ultrasonographic examination and who is undergoing invasive prenatal diagnosis. This test typically can replace the need for fetal karyotype.
- In a patient with a structurally normal fetus who is undergoing invasive prenatal diagnostic testing,

- Most genetic changes identified by chromosomal microarray analysis that typically are not identified on standard karyotype are not associated with increasing maternal age; therefore, the use of this test can be considered for all women, regardless of age, who undergo prenatal diagnostic testing.
- Prenatal chromosomal microarray analysis is recommended for a patient with a fetus with one or more major structural abnormalities identified on ultrasonographic examination and who is undergoing invasive prenatal diagnosis. This test typically can replace the need for fetal karyotype.
- In a patient with a structurally normal fetus who is undergoing invasive prenatal diagnostic testing,

- 那些核型无法检测的染色体小片段改变与孕妇年龄无关，所以Microarray适用于所有需要产前诊断的孕妇人群
- 对于胎儿存在一个或多个超声结构异常的情形，应该建议Microarray检查，并可取代核型分析检测
- 对于不存在胎儿结构异常但需要产前诊断的情形，可以选择核型分析或microarray检测

国内外其他相关专家共识／指南



SOGC–CCMG JOINT TECHNICAL UPDATE

No. 270, December 2011

Use of Array Genomic Hybridization Technology in Prenatal Diagnosis in Canada

This technical update has been prepared by the Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada (SOGC) and the Prenatal Diagnosis Committee of the of the Canadian College of Medical Geneticists (CCMG) and approved by the Executive of the SOGC.

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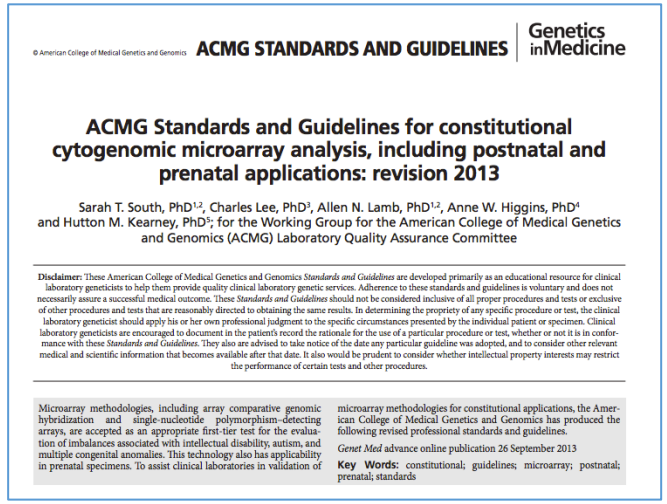
Abstract

Objective: To summarize for obstetrical care providers the current literature on array genomic hybridization in prenatal diagnosis and to outline the recommendations of the Canadian College of Medical Geneticists regarding the use of this new technology with respect to prenatal diagnosis.

Evidence: PubMed and Medline were searched for articles published in English between 2004 and 2010, using the key words DNA QF-PCR, quantitative fluorescent polymerase chain reaction, fetal chromosomal abnormalities, prenatal diagnosis, array genomic hybridization, fetal structural anomalies, and copy number variants. Results were restricted to systematic reviews, randomized control trials/controlled clinical trials, and observational studies. Searches were updated on a regular basis, and articles were incorporated in the guideline to September 2011. Grey (unpublished) literature was identified through searching the websites of health technology assessment and health technology assessment-related agencies, clinical practice guideline collections, clinical trial registries, and national and international medical specialty societies.

Values: The quality of evidence in this document was rated using the criteria described in the Report of the Canadian Task Force on Preventive Health Care (Table 1).

加拿大妇产科学会 2011年



© American College of Medical Genetics and Genomics **ACMG STANDARDS AND GUIDELINES** | Genetics inMedicine

ACMG Standards and Guidelines for constitutional cytogenomic microarray analysis, including postnatal and prenatal applications: revision 2013

Sarah T. South, PhD[1,2], Charles Lee, PhD[3], Allen N. Lamb, PhD[1,2], Anne W. Higgins, PhD[4] and Hutton M. Kearney, PhD[5]; for the Working Group for the American College of Medical Genetics and Genomics (ACMG) Laboratory Quality Assurance Committee

Disclaimer: These American College of Medical Genetics and Genomics *Standards and Guidelines* are developed primarily as an educational resource for clinical laboratory geneticists to help them provide quality clinical laboratory genetic services. Adherence to these standards and guidelines is voluntary and does not necessarily assure a successful medical outcome. These *Standards and Guidelines* should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results. In determining the propriety of any specific procedure or test, the clinical laboratory geneticist should apply his or her own professional judgment to the specific circumstances presented by the individual patient or specimen. Clinical laboratory geneticists are encouraged to document in the patient's record the rationale for the use of a particular procedure or test, whether or not it is in conformance with these *Standards and Guidelines*. They also are advised to take notice of the date any particular guideline was adopted, and to consider other relevant medical and scientific information that becomes available after that date. It also would be prudent to consider whether intellectual property interests may restrict the performance of certain tests and other procedures.

Microarray methodologies, including array comparative genomic hybridization and single-nucleotide polymorphism–detecting arrays, are accepted as an appropriate first-tier test for the evaluation of imbalances associated with intellectual disability, autism, and multiple congenital anomalies. This technology also has applicability in prenatal specimens. To assist clinical laboratories in validation of microarray methodologies for constitutional applications, the American College of Medical Genetics and Genomics has produced the following revised professional standards and guidelines.

Genet Med advance online publication 26 September 2013

Key Words: constitutional; guidelines; microarray; postnatal; prenatal; standards

美国医学遗传学学会 2013年

·临床指南·

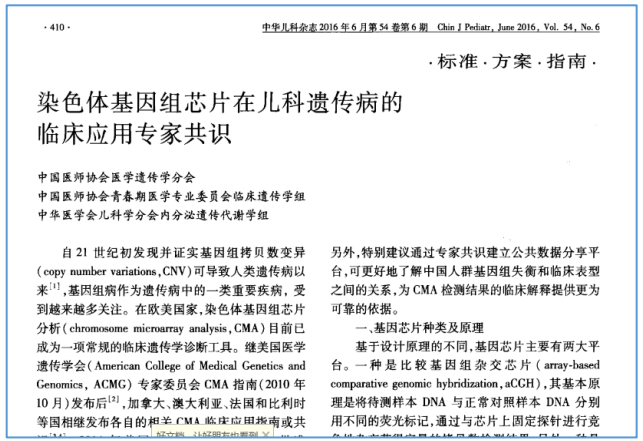
染色体微阵列分析技术在产前诊断中的应用专家共识

染色体微阵列分析技术在产前诊断中的应用协作组

目前，G显带染色体核型分析技术仍然是细胞遗传学产前诊断的"金标准"，但该技术具有细胞培养耗时长、分辨率低以及耗费人力的局限性。包括荧光原位杂交（fluorescence in situ hybridization，FISH）技术在内的快速产前诊断技术的引入虽然具有快速及特异性高的优点，但还不能做到对染色体组的全局分析。染色体微阵列分析（chromosomal microarray analysis，CMA）技术又被称为"分子核型分析"，能够在全基因组水平进行扫描，可检测染色体不平衡的拷贝数变异（copy number variant，CNV），尤其是对于检测染色体组微小缺失、重复等不平衡性重排具有突出优势。根据芯片设计与检测原理的不同，CMA技术可分为两大类，其于微阵

ISCA Consortium推荐将aCGH作为对原因不明的发育迟缓、智力低下、多种体征畸形以及自闭症患者的首选临床一线检测方法[5]。近年来，CMA技术在产前诊断领域中的应用越来越广泛，很多研究也证明了该技术具有传统胎儿染色体核型分析方法所无法比拟的优势。CMA对非整倍体和不平衡性染色体重排的检出效率与传统核型分析方法相同，并具有更高的分辨率和敏感性，且CMA还能发现额外的、有临床意义的基因组CNV，尤其是对于产前超声检查发现胎儿结构异常者，CMA是目前最有效的遗传学诊断方法[5-6]。基于上述研究结果，不少学者认为，CMA技术有可能取代传统的核型分析方法，成为产前遗传学诊断的一线技术。但到目

中华妇产科杂志 2014年



·标准·方案·指南·

染色体基因组芯片在儿科遗传病的临床应用专家共识

中国医师协会医学遗传学分会
中国医师协会青春期医学专业委员会临床遗传学组
中华医学会儿科学分会内分泌遗传代谢学组

自21世纪初发现并证实基因组拷贝数变异（copy number variations，CNV）可导致人类遗传病以来[1]，基因组病作为遗传病中的一类重要疾病，受到越来越多关注。在欧美国家，染色体基因组芯片分析（chromosome microarray analysis，CMA）目前已成为一项常规的临床遗传学诊断工具。继美国医学遗传学会（American College of Medical Genetics and Genomics，ACMG）专家委员会CMA指南（2010年10月）发布后[2]，加拿大、澳大利亚、法国和比利时等国相继发布各自的相关CMA临床应用指南或共

另外，特别建议通过专家共识建立公共数据分享平台，可更好地了解中国人群基因组失衡和临床表型之间的关系，为CMA检测结果的临床解释提供更为可靠的依据。

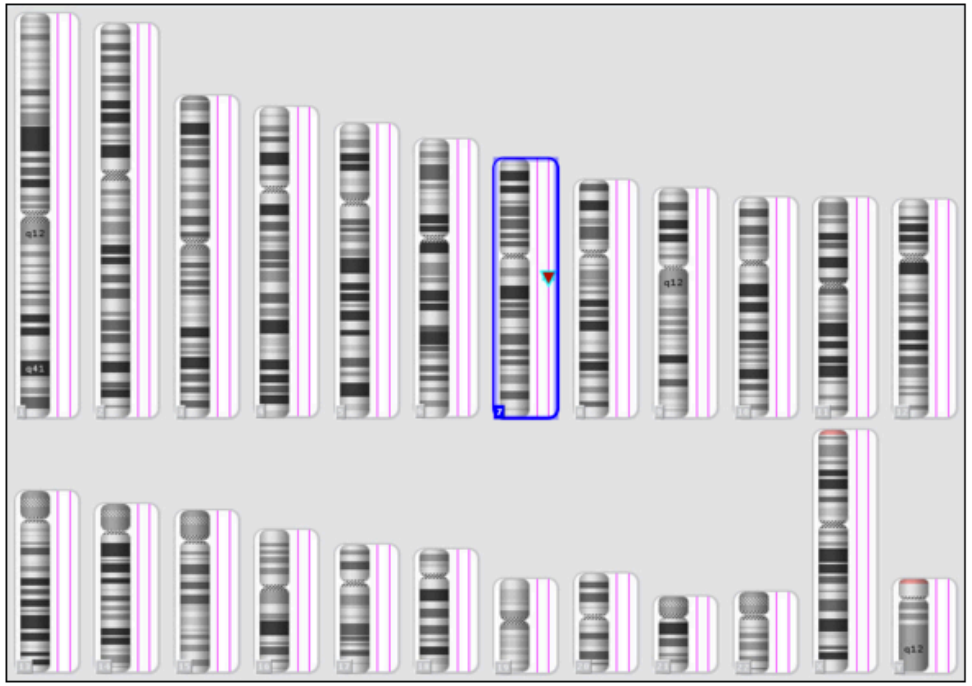
一、基因芯片种类及原理

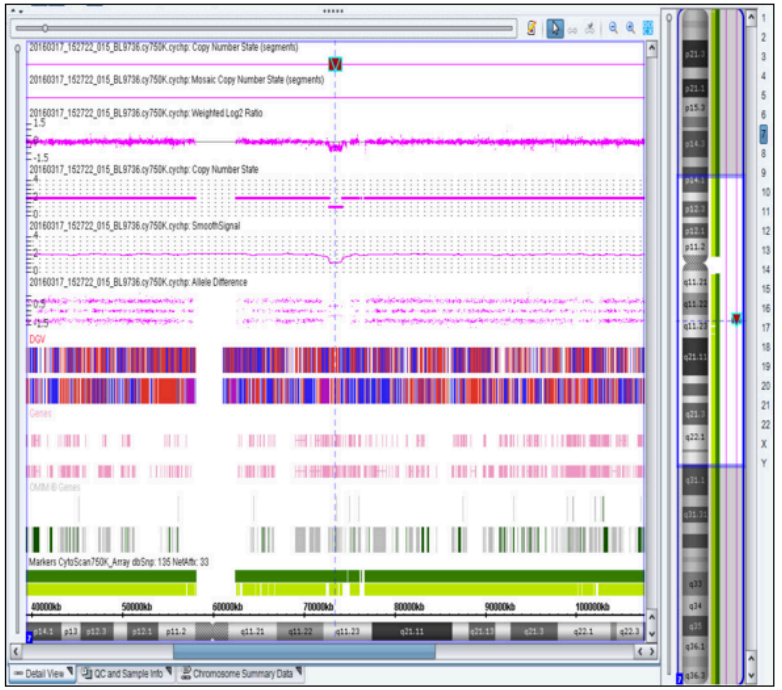
基于设计原理的不同，基因芯片主要有两大平台。一种是比较基因组杂交芯片（array-based comparative genomic hybridization，aCGH），其基本原理是将待测样本DNA与正常对照样本DNA分别用不同的荧光标记，通过与芯片上固定探针进行竞

中华儿科杂志 2016年

Microarray诊断染色体片段缺失／重复的一些挑战

10岁患儿，智力低下就诊，并咨询下一胎健康妊娠的可能性

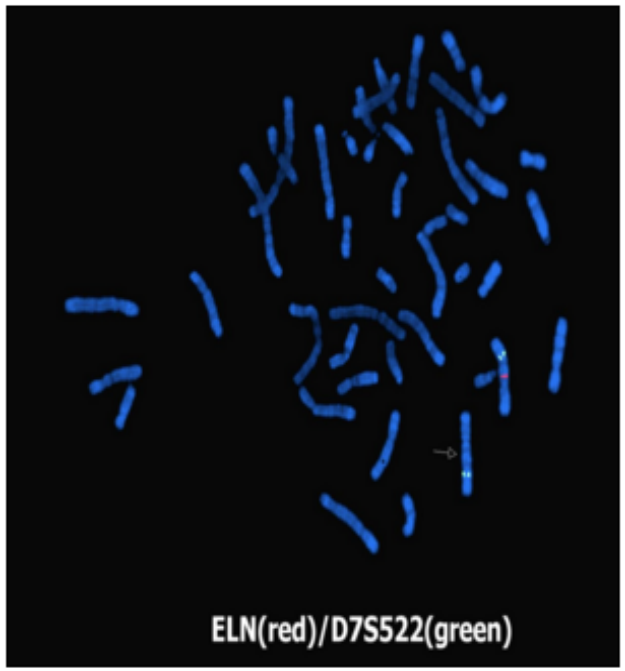




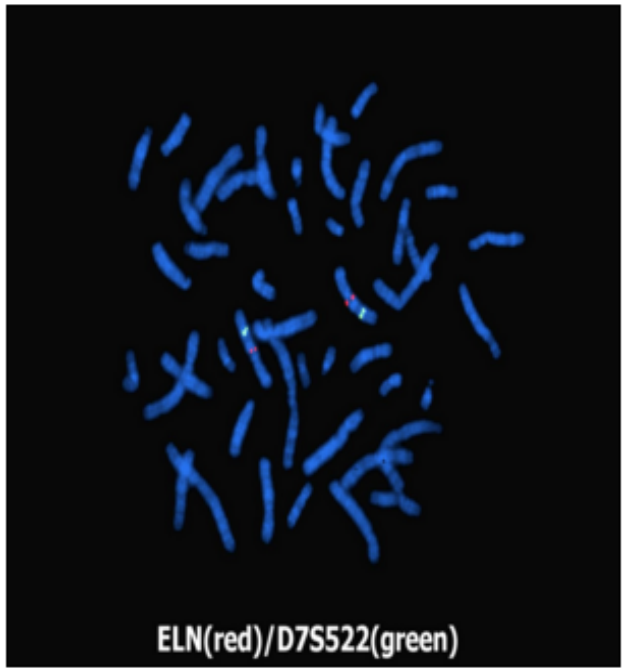
7号染色体7q11.23区段存在1.4Mb片段的缺失，内含ELN, LIMK1, GTF2I, GTF2IRD1等22个OMIM基因，涉及Williams-Beuren综合征(Williams-Beuren Syndrome)疾病区域

临床表型包括身材矮小，颅面部异常，智力障碍，先天性心脏病等

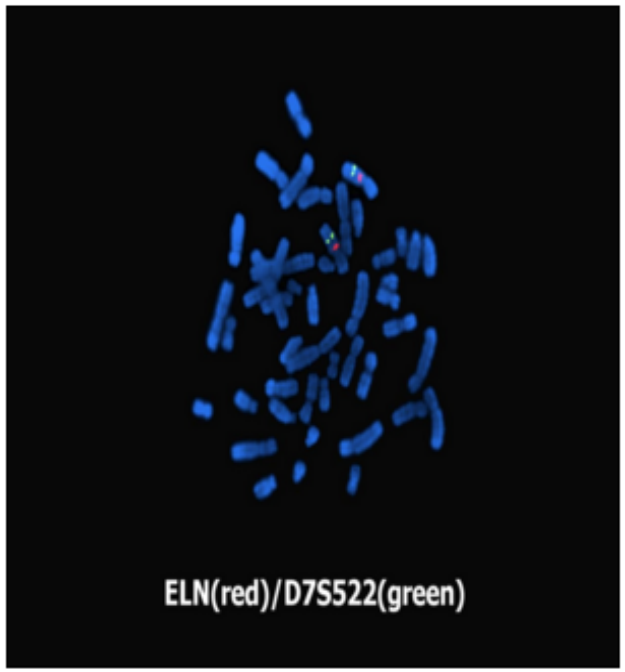
家系FISH验证



患儿：缺失



母亲：正常

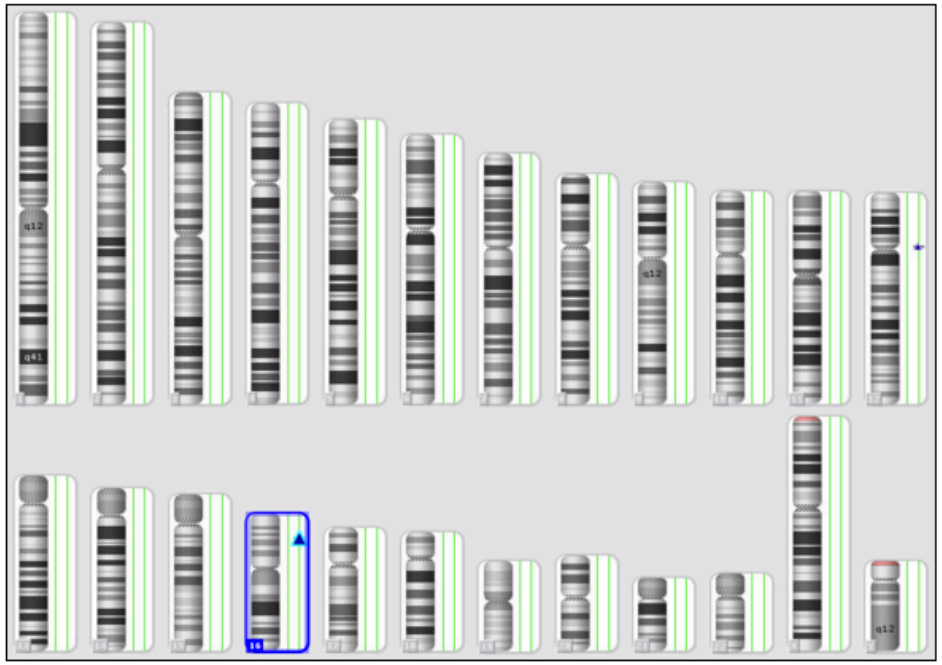


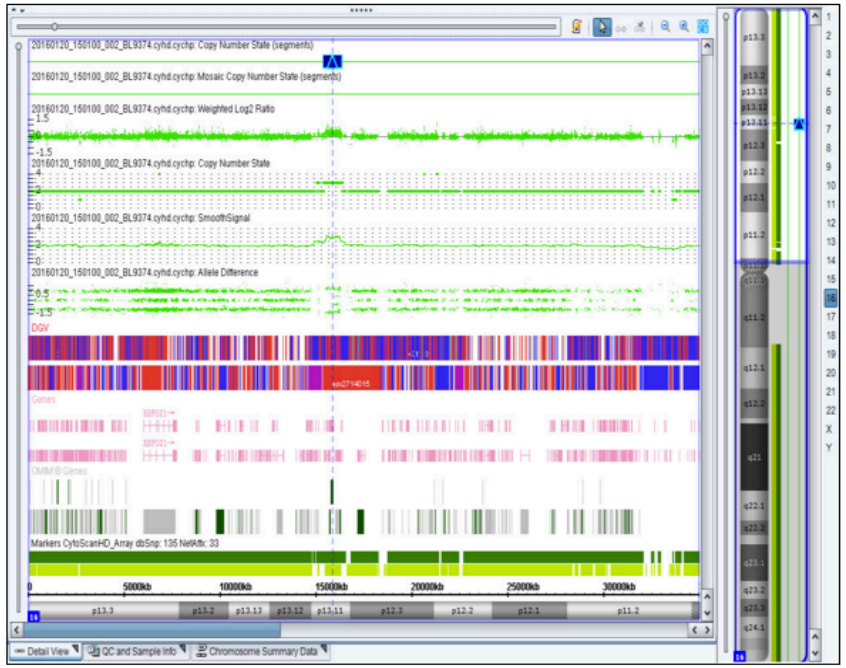
父亲：正常

新生突变，再发风险小于1%

然而并不是所有的微缺失／重复都是新发的

7岁患儿，疑诊自闭症，伴有认知能力的下降

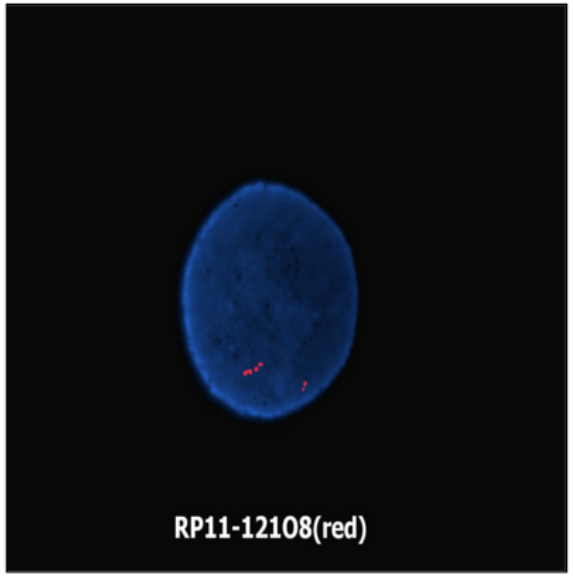


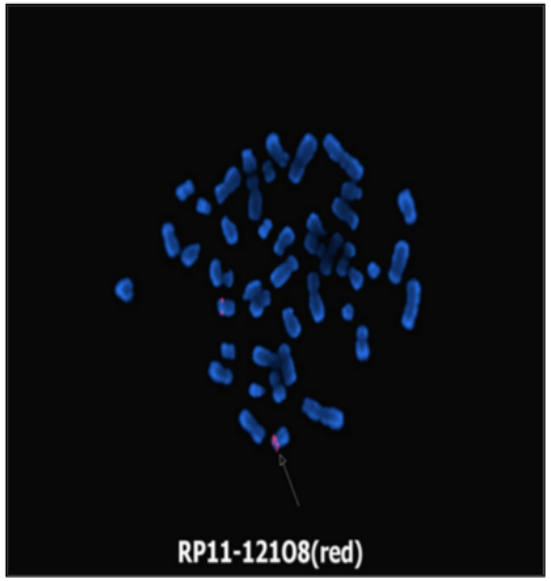


16号染色体16p13.11区段存在965.3Kb片段的重复，内含NDE1, MYH11, ABCC6等6个OMIM基因，该片段位于神经认知敏感区域。
已有研究报道该片段重复与复发性16p13.11重复综合征(16p13.11 recurrent microduplication)疾病相关，临床表型包括智力低下，脑发育异常等

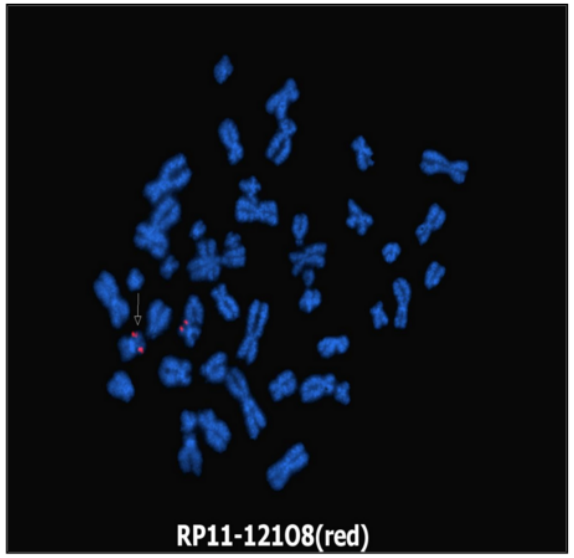
FISH验证

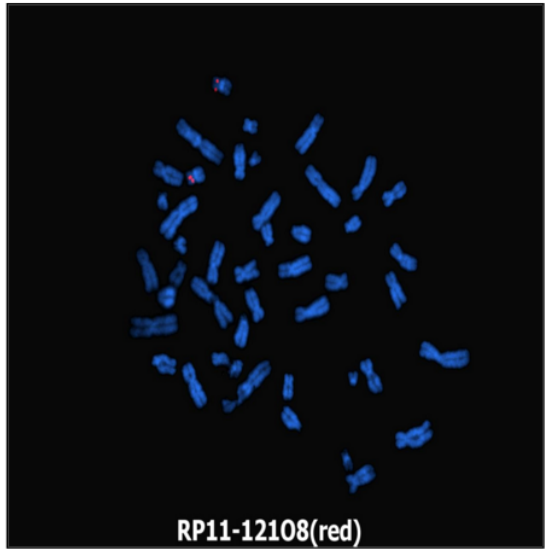
患儿：重复





母亲：携带

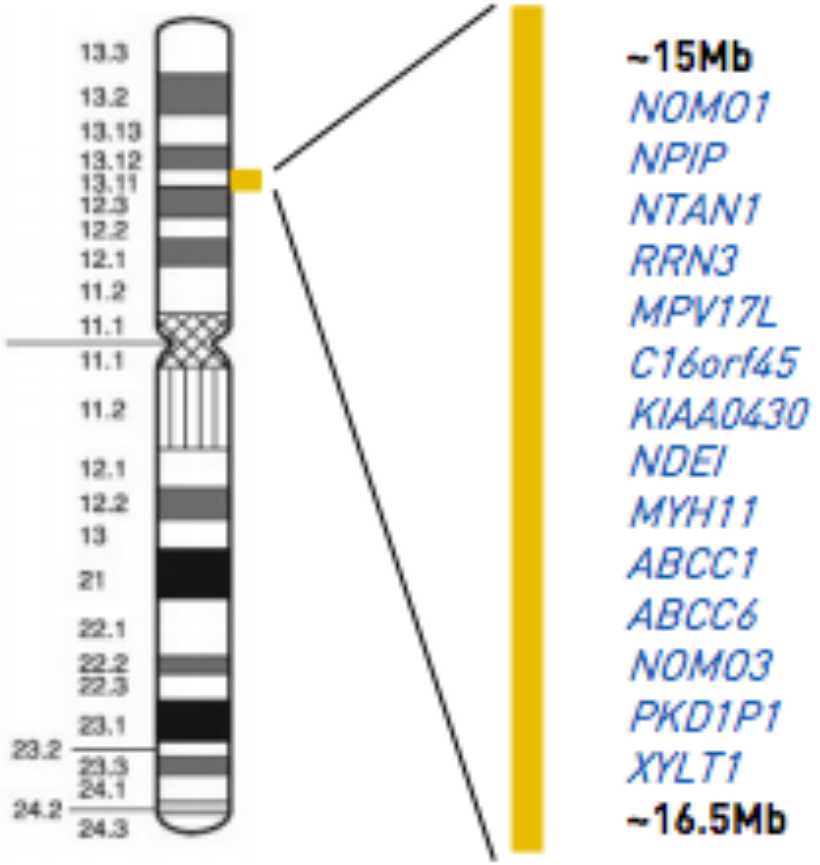




父亲：正常

16P13.11重复综合征

- 近年来新认识的一种以神经系统发育异常为主要表现的微重复综合征
- 多为LCRs介导的非同源染色体重组所导致
- 新近的文献报道在智力发育异常的人群中发病率为0.73%
- 主要疾病表现为智力发育障碍，自闭症，癫痫，精神分裂症，注意力缺失伴焦虑异常等，以及成人发病的胸主动脉瘤等
- 有部分的患者该重复异常遗传自表型相对正常的亲代

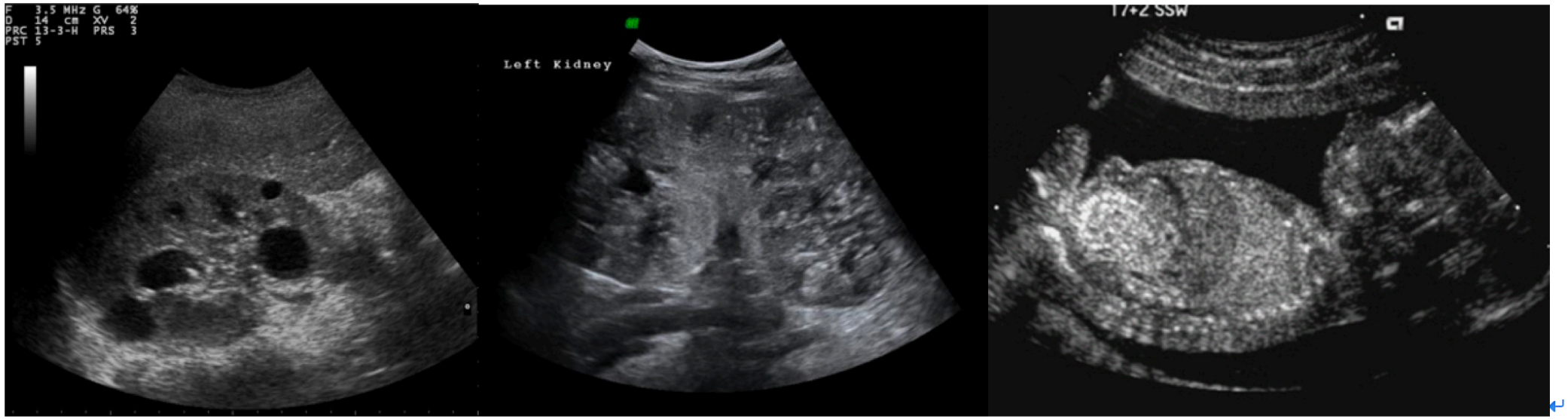


3例17q12微缺失综合征的病例

- 孕妇1，32岁，G1P0，NT检测及唐氏筛查均低危。孕22周胎儿系统超声提示双肾多发囊肿最大直径1.2cm，双侧肾盂增宽1.1cm，双侧脑室增宽1.2cm左右，单脐动脉。为进一步诊断于孕25^{+2}周行脐血穿刺，送检核型分析及CMA

- 孕妇2，27岁，G1P0，NT检测正常，唐氏筛查风险低危。28周超声检查双侧肾回声增强，3周后再次复查仍提示双侧肾实质回声增强，32^{+6}周行脐血穿刺进一步遗传诊断

- 孕妇3，33岁，G2P0，既往本人有多年的右侧肾囊肿病史，无糖尿病，肾功能异常或神经系统认知功能异常。自述2年前妊娠第一胎孕中期超声提示胎儿双侧肾脏结构不清，羊水极少引产，未行遗传诊断。本次妊娠18周超声检测提示双侧肾实质回声增强，羊水量正常，为行进一步遗传诊断行羊膜腔穿刺进行核型分析及CMA检测。

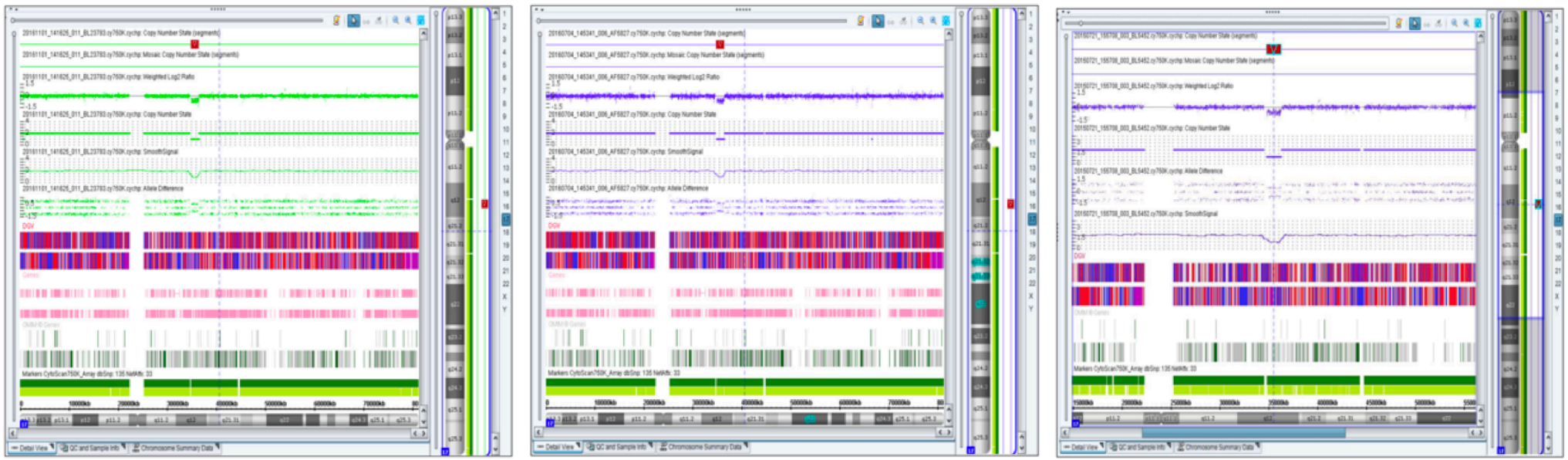


A B C

图 1: 3 例胎儿的肾脏异常表现　　A. 胎儿 1 多囊性改变；B. 胎儿 2 肾脏回声增强；C. 胎儿 3 肾脏回声增强

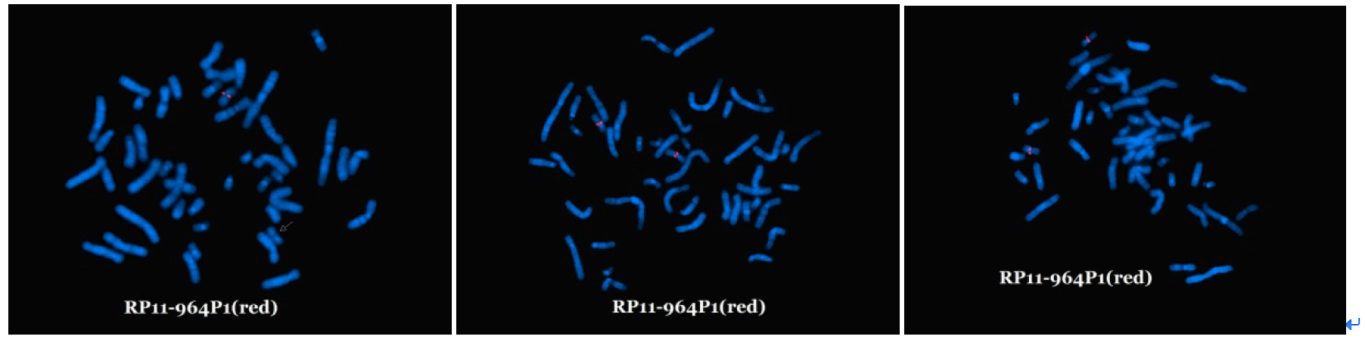
表 1 3 例胎儿 CMA 诊断结果及亲代验证结果

	SNP Array 结果	片段大小	父母验证来源
胎儿 1	arr[hg19]17q12(34,822,465-36,404,555)x1	1.5Mb	新发突变
胎儿 2	arr[hg19]17q12(34,822,465-36,425,336)x1	1.6Mb	新发突变
胎儿 3	arr[hg19]17q12(34,822,465-36,243,365)x1	1.421Mb	遗传自母亲
胎儿 3 母亲	arr[hg19]17q12(34,460,443-36,370,997)x1	1.9Mb	未验证



A B C

图 3 3 例胎儿的 17q12 缺失染色体详图 A. 胎儿 1；B. 胎儿 2；C. 胎儿 3



A　　B　　C

图 4　胎儿 1 的染色体 17q12 缺失经过亲代 FISH 验证证明为新发突变　A. 胎儿；B. 母亲；C. 父亲

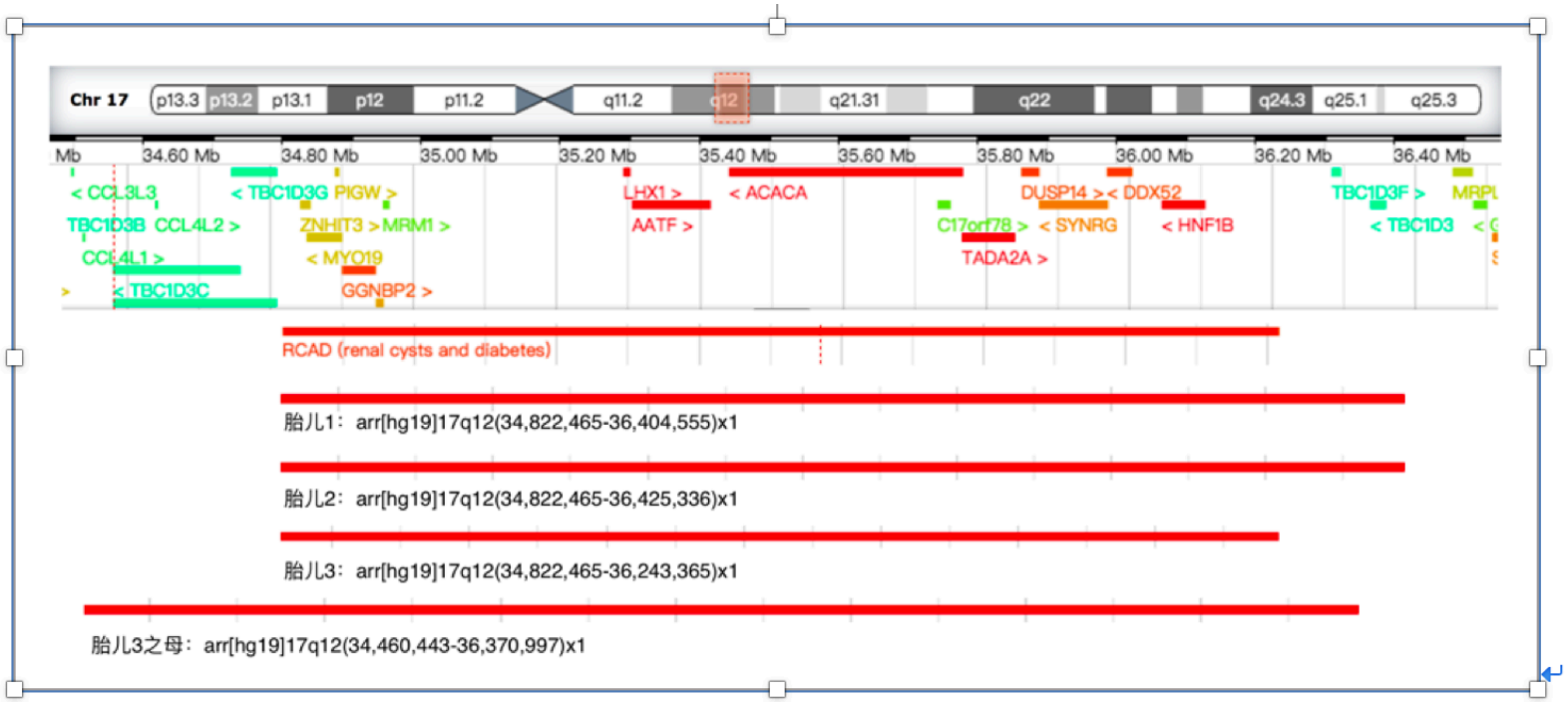
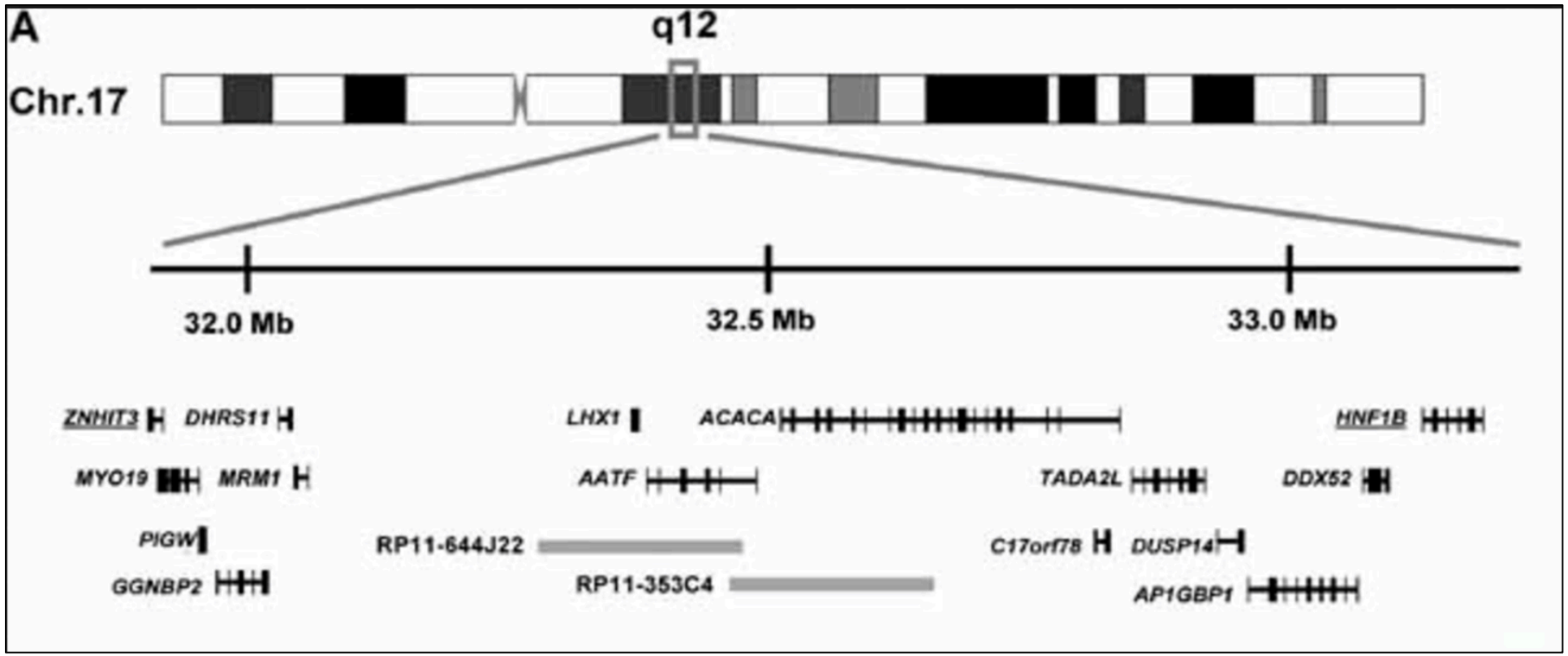


图 5 3 例胎儿的 17q12 染色体缺失区域对比图

最上面红线为典型的微缺失片段区域，其下 4 条红线代表胎儿 1-3 及胎儿 3 之母的染色体缺失区域

17q12微缺失综合征的区域与基因

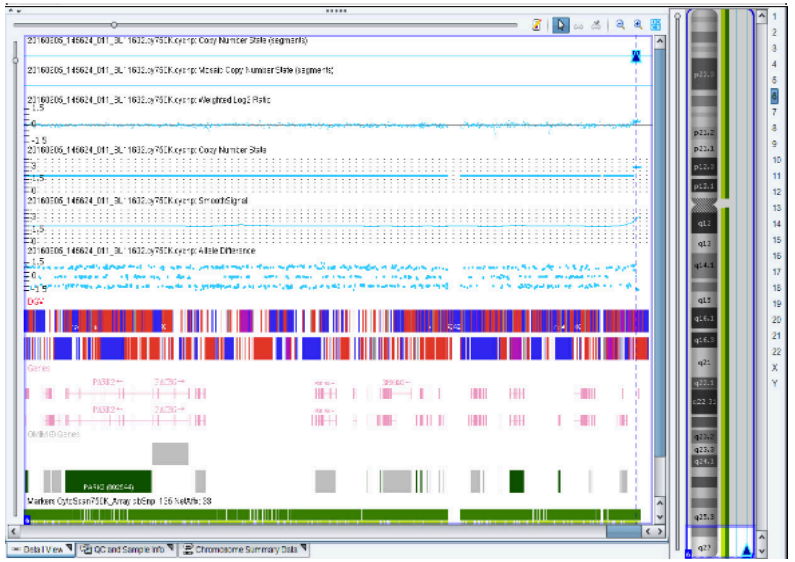


遗传咨询的困难

- 病例3的胎儿17q12的缺失来源自母亲的遗传，说明孕妇本人也是一个17q12缺失综合征的患者。而胎儿1-2经过父母验证证实是一个新发的突变。
- 对于胎儿3，由于17q12微缺失综合征的表型多种多样，轻重不一，孕妇本人目前仅出现一侧小的肾囊肿，没有糖尿病的发生或神经认知方面的异常是可以理解的，但这并不排除她将来会出现这些方面的表型，或已有表型的加重，也不能完全肯定预测胎儿3的将来预后会和孕妇本人一样比较轻微
- 所以当产前诊断发现胎儿存在17q12微缺失综合征时，对于生后的表型预测和咨询是非常有挑战的。
- 开展亲代验证的工作是非常必要的，它不但可以明确来源，判断染色体变异的发生机制，有助于胎儿生后表型的初步估计，也对于诊断症状轻微的亲代患病以及其后的随访和治疗有重要的意义

病例 意外发现

- 徐XX，33岁，G2P1，因12周NT检测3.2mm，行羊水穿刺诊断
- 核型分析结果正常
- Microarray检测胎儿在6号染色体6q27区段存在148.1Kb片段的重复，内含TBP等3个OMIM基因，该基因内三核苷酸重复(CAG)n异常与常染色体显性遗传的脊髓小脑共济失调(Spinocerebellar ataxia)疾病相关，临床表型包括小脑萎缩，眼球震颤，吞咽困难，肌张力障碍，动作迟缓，帕金森症，痴呆，癫痫等
- 但也有正常人群携带该种异常的报道
- 孕妇本人的microarray检测证实遗传自孕妇本人， 6号染色体6q27区段存在114.2Kb片段的重复
- 由于脊髓小脑共济失调多于30岁之后隐匿起病，建议神经科进一步随诊



孕妇

胎儿

总结

- 分子遗传诊断技术在产前诊断的应用给我们打开了一扇窗
- 许多新的染色体疾病尤其是染色体微小片段的改变在产前阶段得以诊断
- 但同时也对当前的产前诊断体系带来了变革
- 适宜的指证，检测结果正确的解读和处理是目前最需要解决的临床问题
- 也需要产前遗传咨询的大夫有更宽广的相关知识储备和咨询技能
- 这些都需要大量的病例积累经验，以及相互分享学习来提高
- 为了实现更精准的产前诊断结果，围绕分子遗传诊断技术的临床应用和遗传咨询也非常关键，这对于未来产前诊断中心技术体系的搭建提出了更高的要求

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