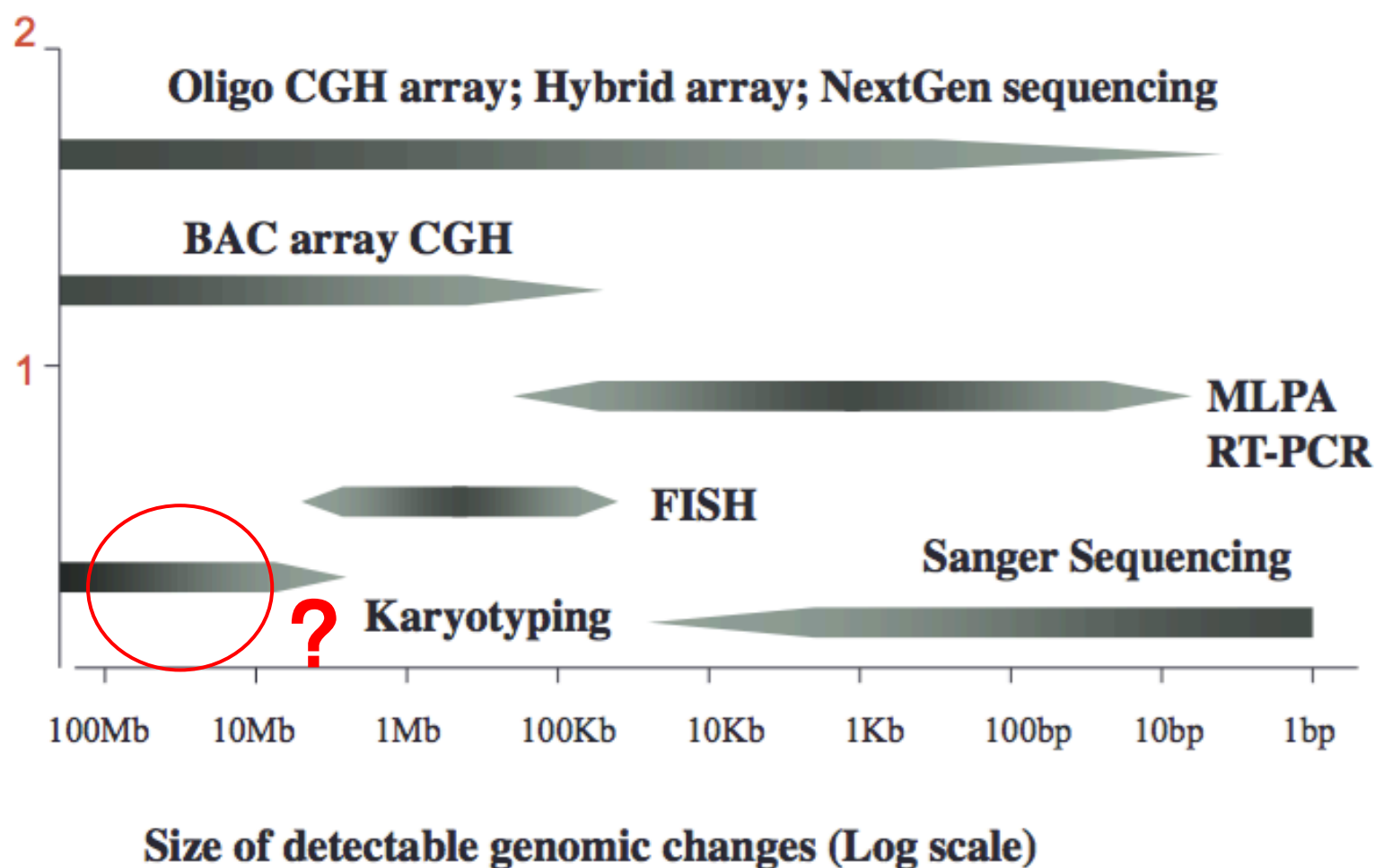


产前／儿科分子遗传诊断的病例讨论

北京协和医院妇产科 蒋宇林

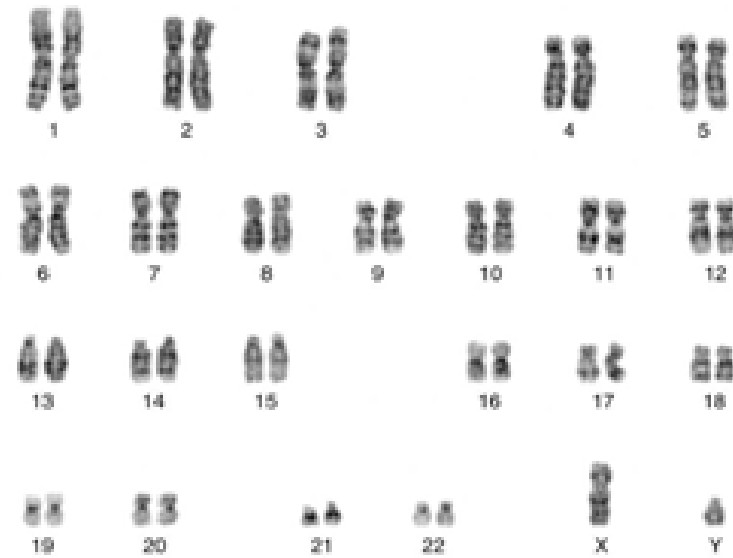
人类基因突变谱系与检测方法



普通 / 高分辨核型分析



高分辨G显带650条带以上



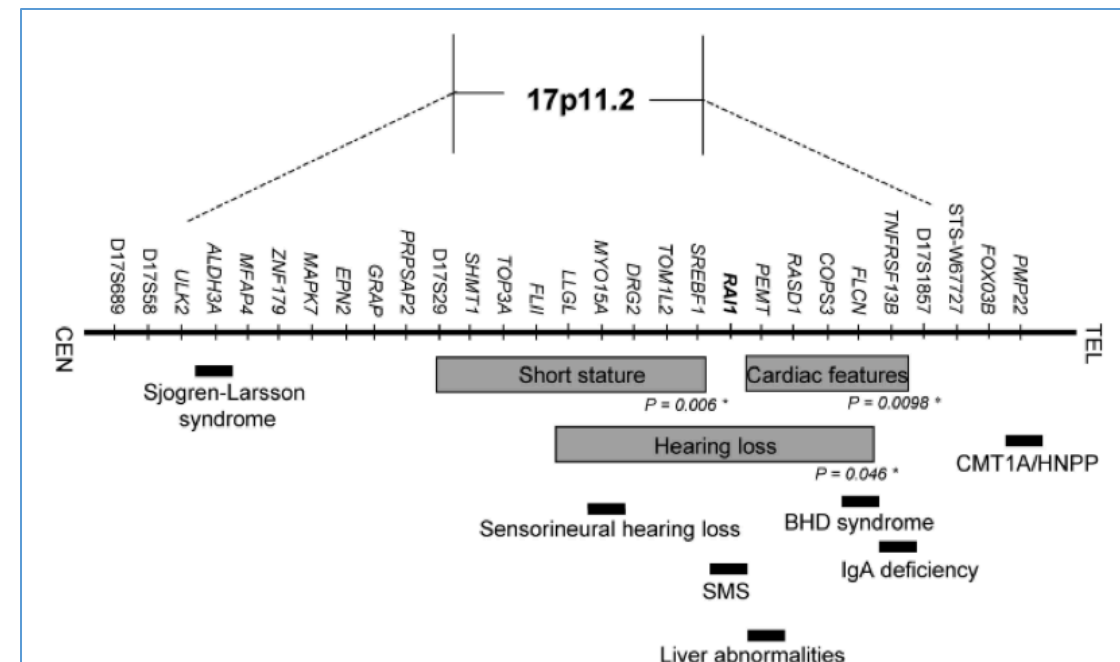
常规G显带340条带

遗传诊断 — 核型分析就够了吗？



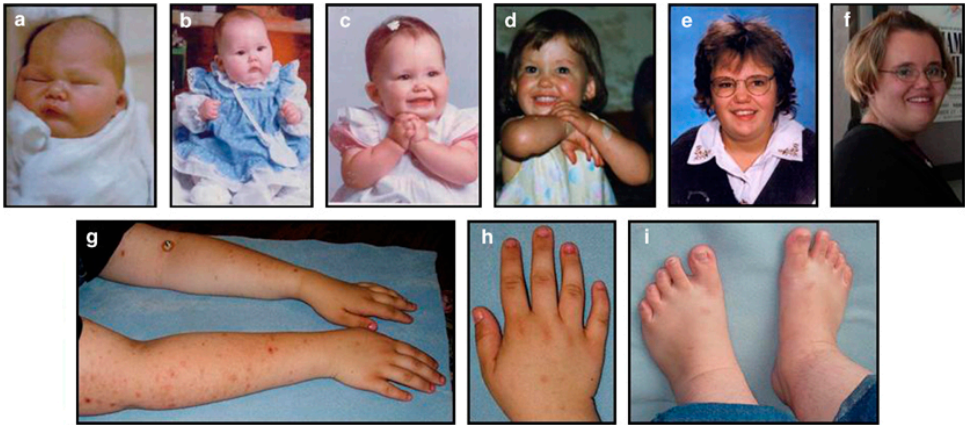
- 女性患儿15岁，身高1.44米，智力低下，语言能力弱，能回答姓名等简单问题，容易兴奋，在诊室内又唱又跳
- 面部异常:外眦上斜,巨舌,外吐
- 睡眠:现在每天仅仅睡眠2-3小时，时睡时醒
- 手:双手有咬伤伤痕。暴躁时抓人打人,自己抓头发、抓耳,指甲损伤严重。

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

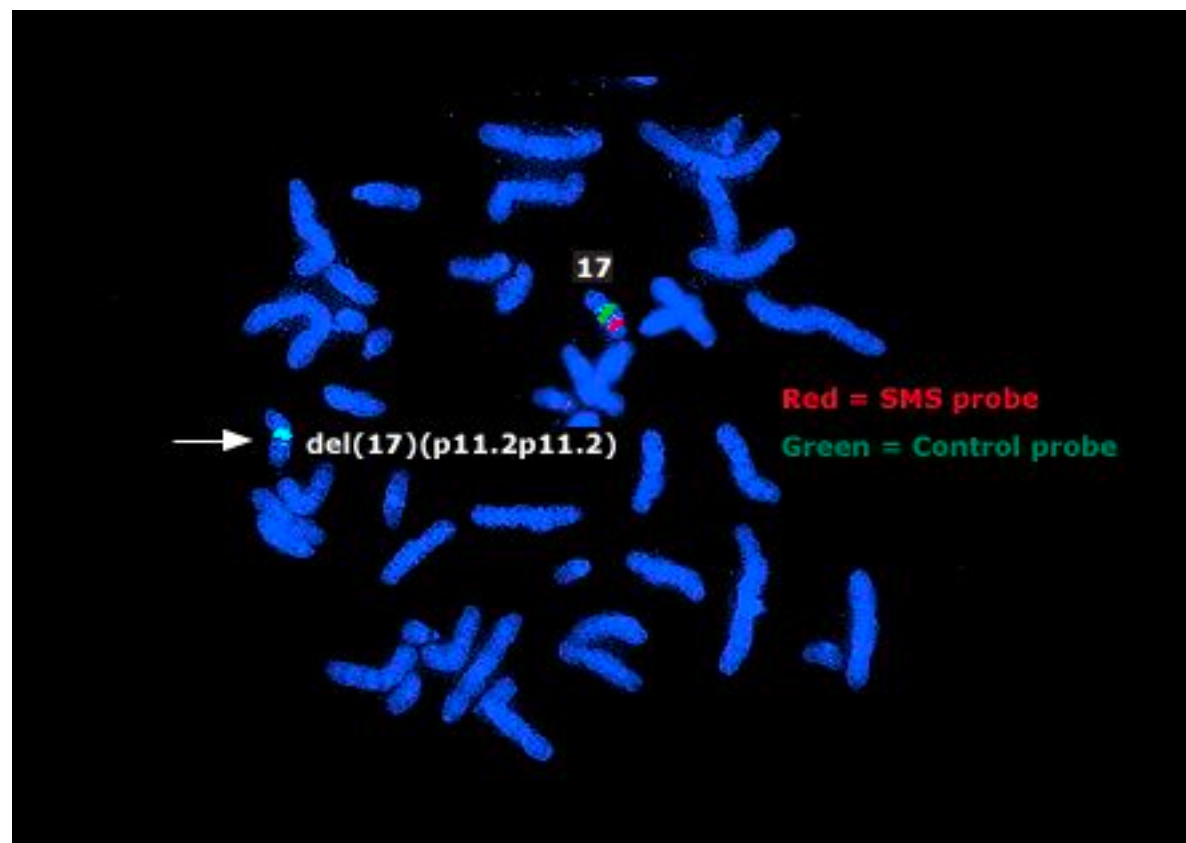


- ▶ 较常见的微缺失综合征，发生率1/25000
- ▶ 17p11.2缺失导致
- ▶ 产前临床表现：无报道
- ▶ 产后临床表现：发育迟缓、智力迟滞、行为异常等
 - 小头畸形，联眉，内眦赘皮，
 - 入睡困难，易激惹、注意力低下，自残行为，痛阈低下，剔甲癖等

| 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) |

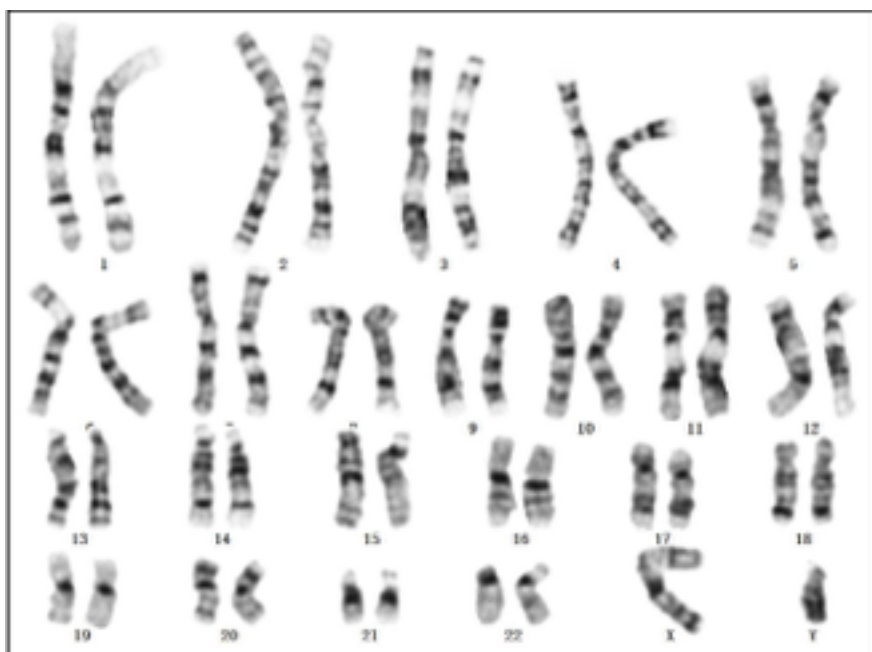


如何诊断 — 荧光原位杂交技术 (FISH)

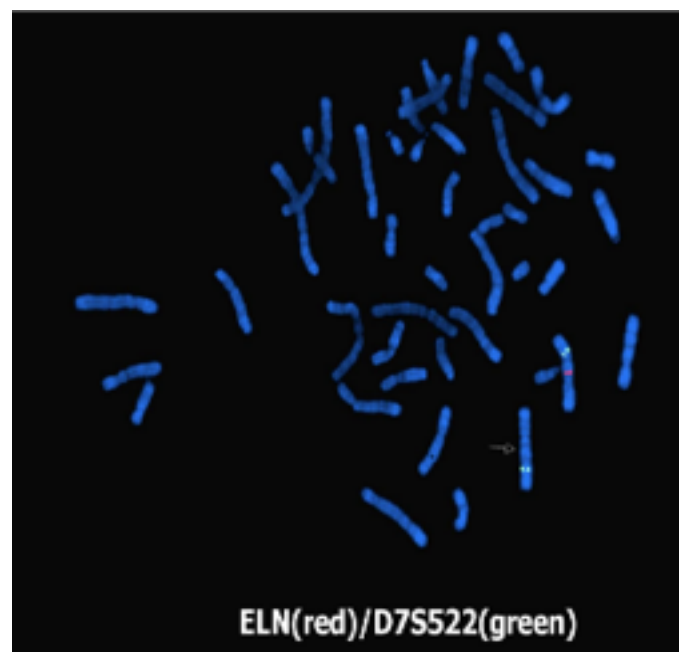


5岁男孩，智力和体格发育迟缓，幼年时做过心脏室缺修补术，怀疑是某种染色体病

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



46, XY



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

Williams-Beuren综合征(WBS)-7q11.23



- ▶ 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



Williams综合征：一个关于NIPT让人后怕的病例

- 刘XX，25岁，先天性智障，能简单回答问题
- 曾生育一个同样表现的智障男孩，目前5岁
- 本次再孕，15周外院就诊
- 外院查NIPT-plus：提示未见异常
- 外院医生告知“可以继续妊娠”
- 孕18周来协和门诊咨询
- 查本人外周血 7q11.23 (72692112-74154209) X1
- 诊断为Williams综合征患者，有50%几率生育同样的患儿
- 孕21周进行羊水穿刺，核型和芯片诊断胎儿正常
- 再次单盲送样NIPT-plus，告知“结果正常”

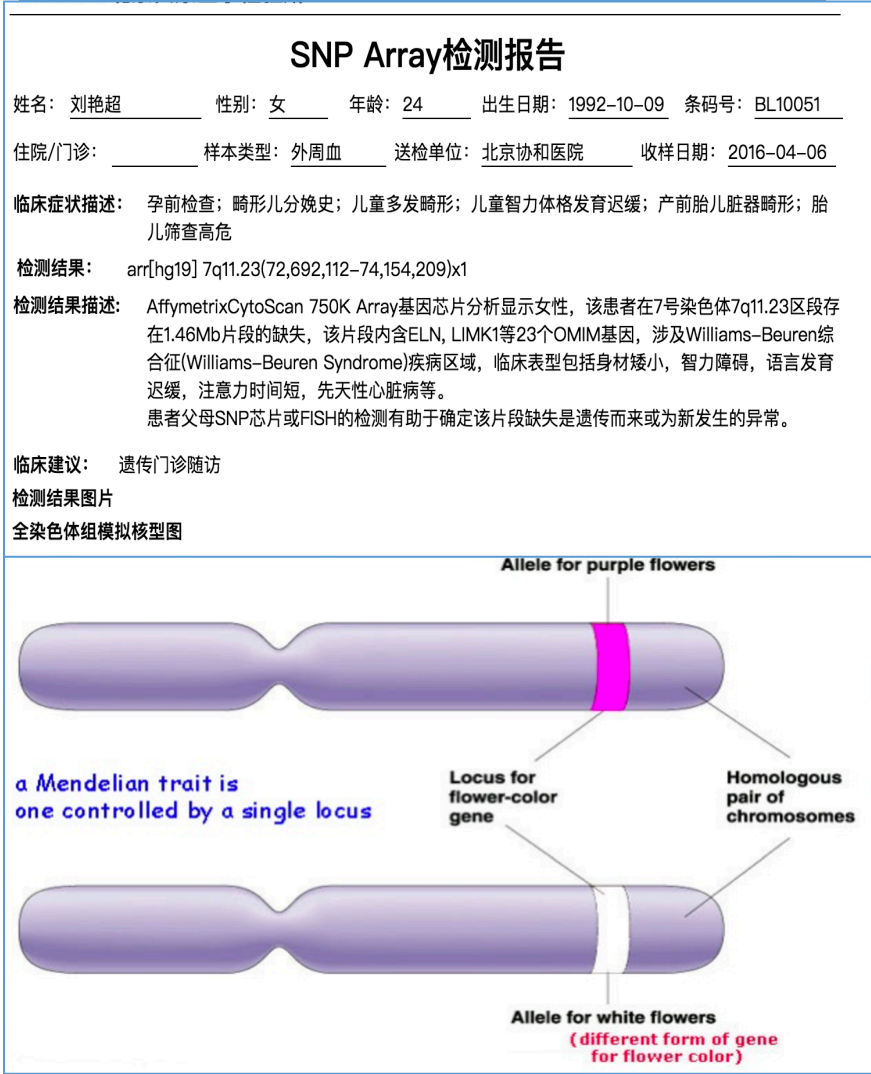


TABLE 1: Description of the aneuploidies and microdeletion syndromes included in the Prenatal BoBS kit.

| 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

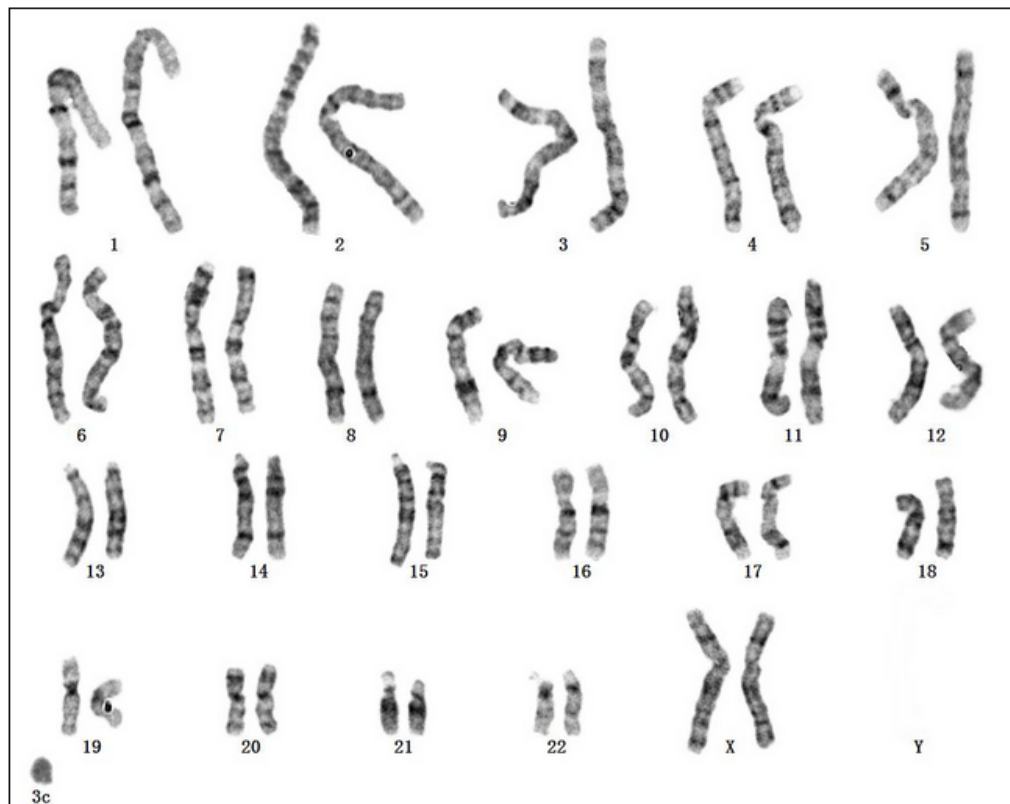
常见的微缺失综合征

| 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 |

FISH检测就能解决一切吗？

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]

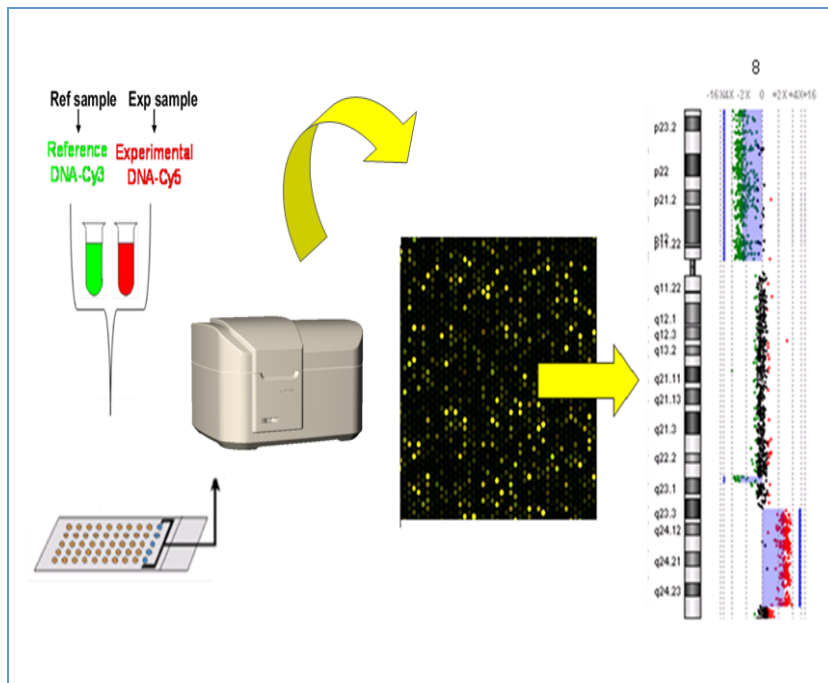
已排除了夫妇双方的染色体异常 (核型分析均正常)



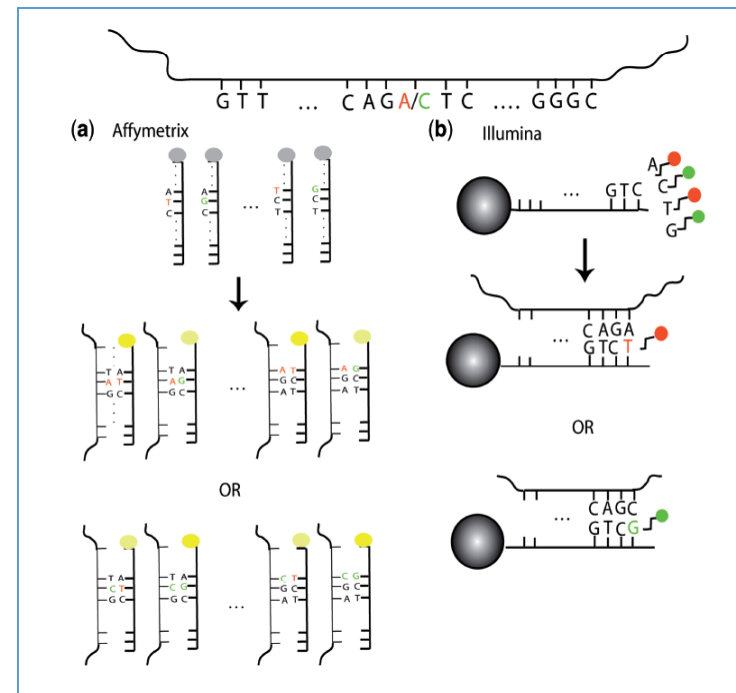
- Marker染色体指异常衍生的染色体, 往往来源不明
- 有时带有重复的染色体片段, 有时不含有关键性的基因
- 有时在正常人群中发现而没有表型
- 临床意义不明
- 需要明确衍生染色体的来源, 含有的片段具体位置才能准确解析
- 该病例中由于没有目标性的判断, 难以从FISH检测中找到原因

染色体微阵列分析 (chromosomal microarray analysis, CMA)

基于CGH的平台

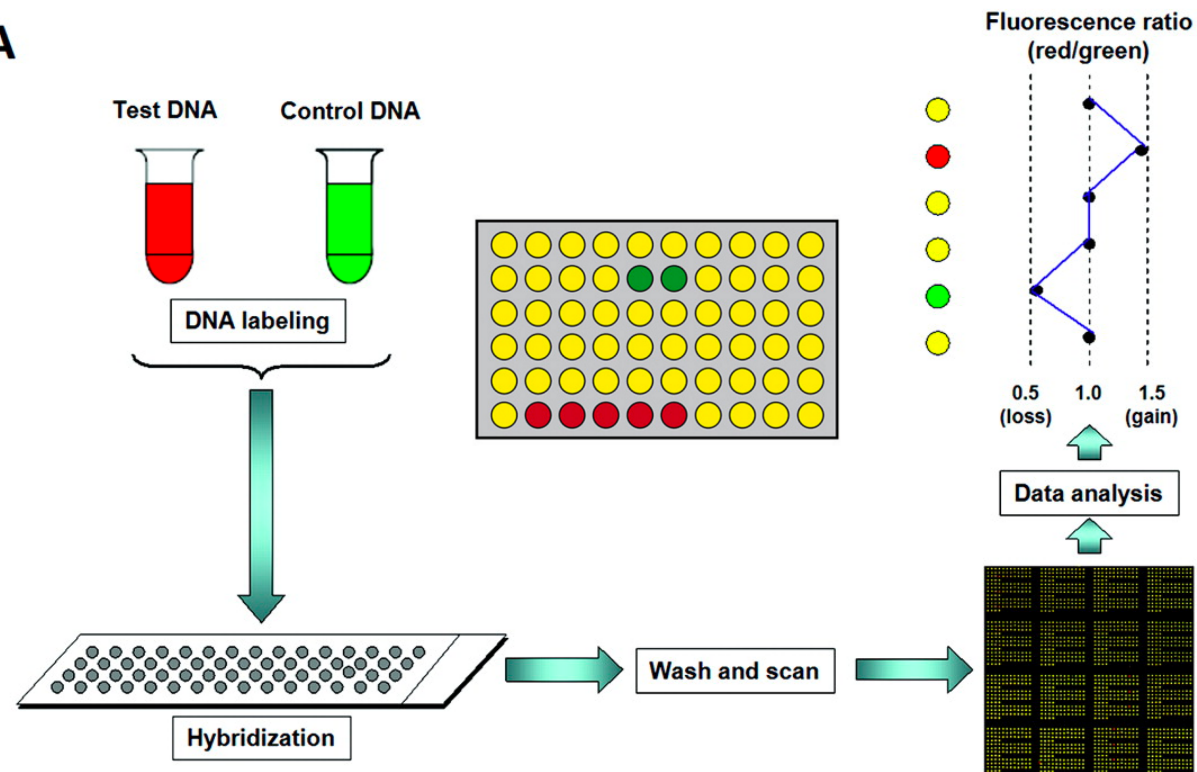


基于 SNP的平台

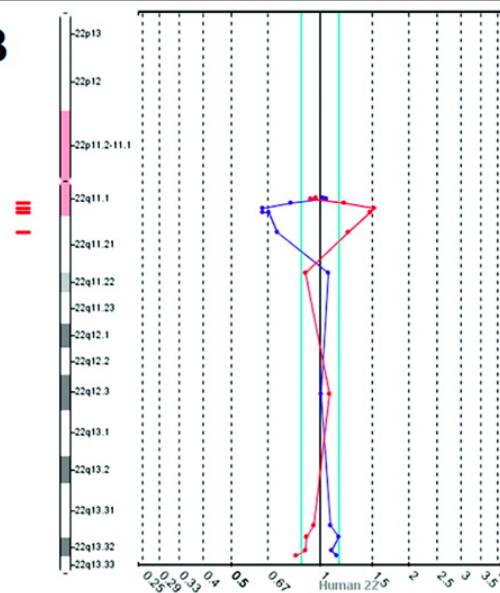


Array CGH原理

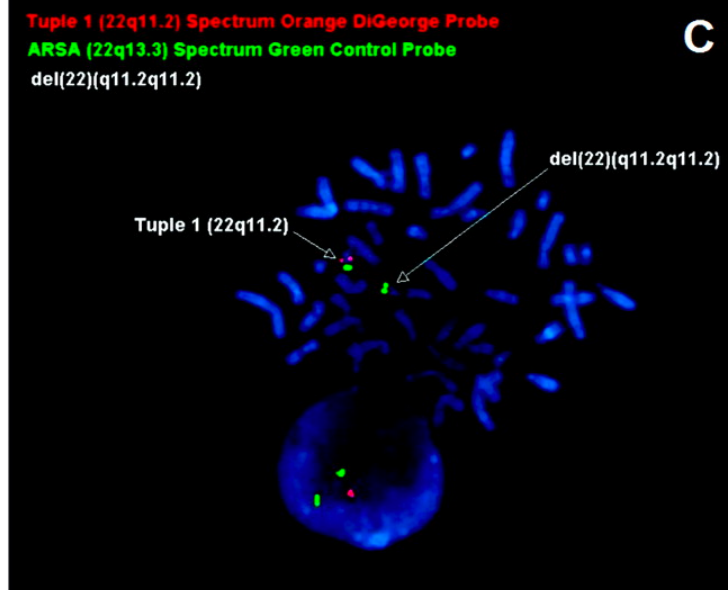
A



B



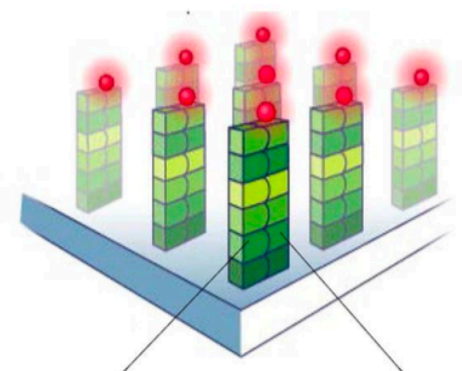
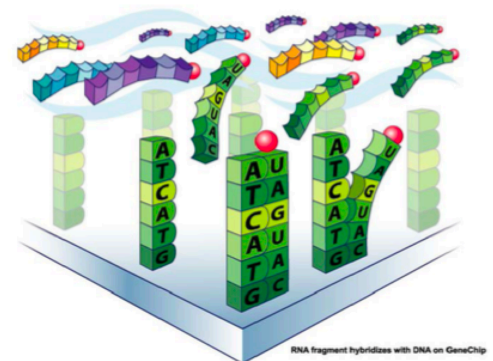
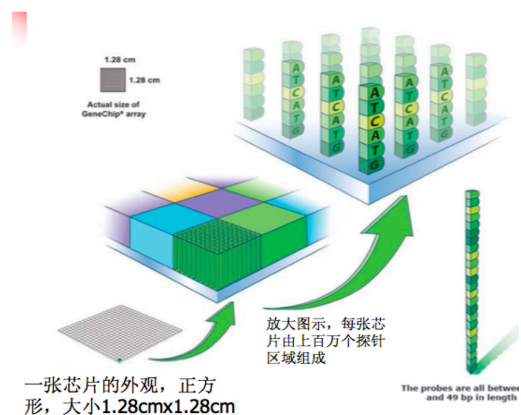
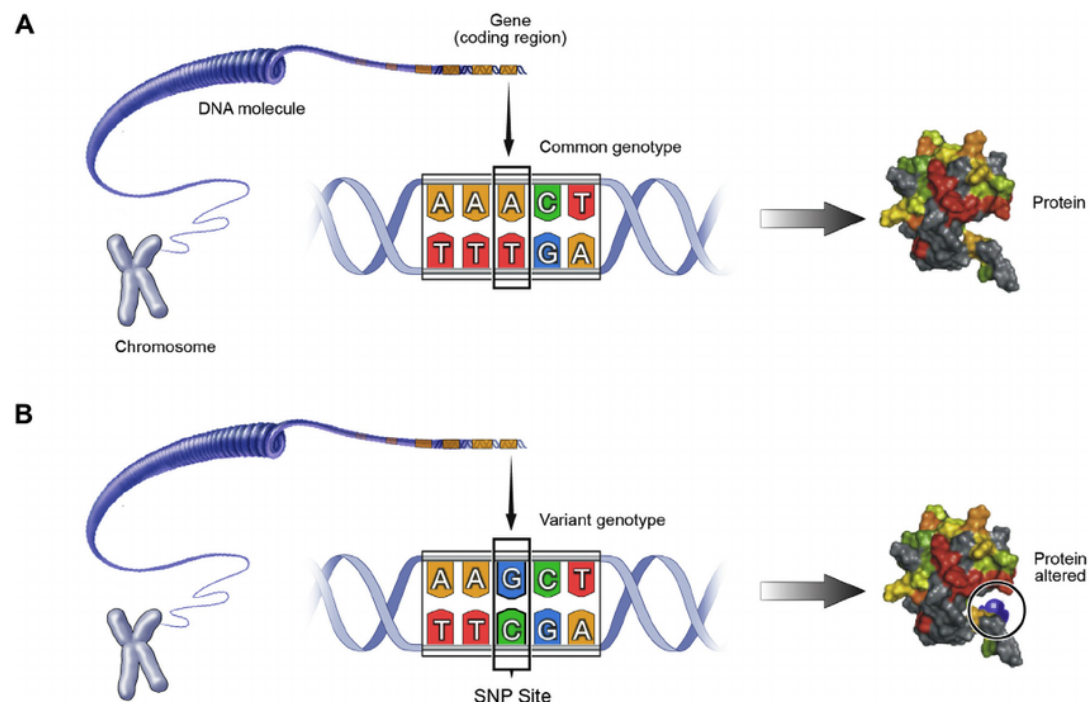
C



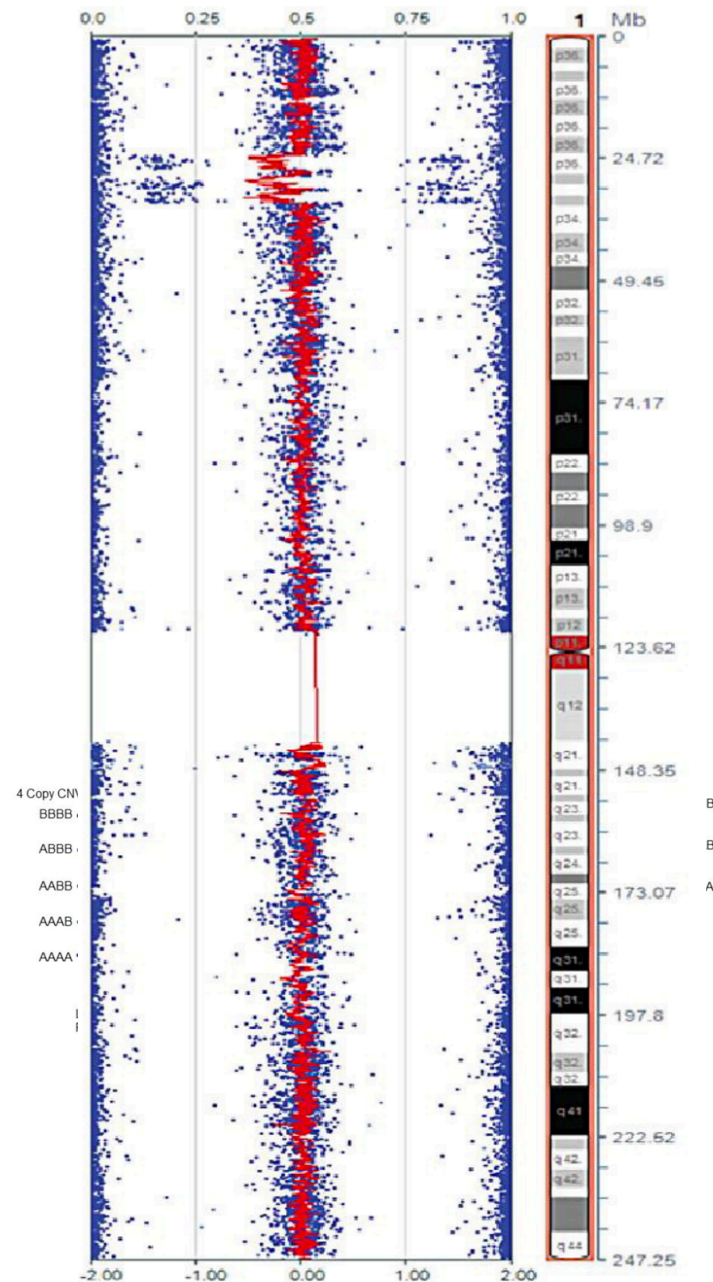
SNP Array原理

核型分析的分辨率 10Mb以上

CMA分析的分辨率 100K以上



Chrom 1
B Allele Freq



Affymetrix SNP-Microarray 系统



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DECEMBER 6, 2012

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Chromosomal Microarray versus Karyotyping for Prenatal Diagnosis

Ronald J. Wapner, M.D., Christa Lese Martin, Ph.D., Brynn Levy, M.Sc.(Med.), Ph.D., Blake C. Ballif, Ph.D., Christine M. Eng, M.D., Julia M. Zachary, Melissa Savage, M.S., Lawrence D. Platt, M.D., Daniel Saltzman, M.D., William A. Grobman, M.D., M.B.A., Susan Klugman, M.D., Thomas Scholl, Ph.D., Joe Leigh Simpson, M.D., Kimberly McCall, B.S., Vimla S. Aggarwal, M.B., B.S., Brian Bunke, B.S., Odelia Nahum, M.Sc., Ankita Patel, Ph.D., Allen N. Lamb, Ph.D., Elizabeth A. Thom, Ph.D., Arthur L. Beaudet, M.D., David H. Ledbetter, Ph.D., Lisa G. Shaffer, Ph.D., and Laird Jackson, M.D.

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



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



Society for
Maternal-Fetal
Medicine

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NUMBER 162, MAY 2016

(Replaces Practice Bulletin Number 88, December 2005
(See also Practice Bulletin Number 163, Screening for Fetal Aneuploidy, May 2016))

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

核型分析或microarray可以适用于任何一例产前诊断的病例

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

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

without FISH analysis. For 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

diagnostic somal m cells, it is or stillbir

Some mations a Increasing tions is av ings, such common

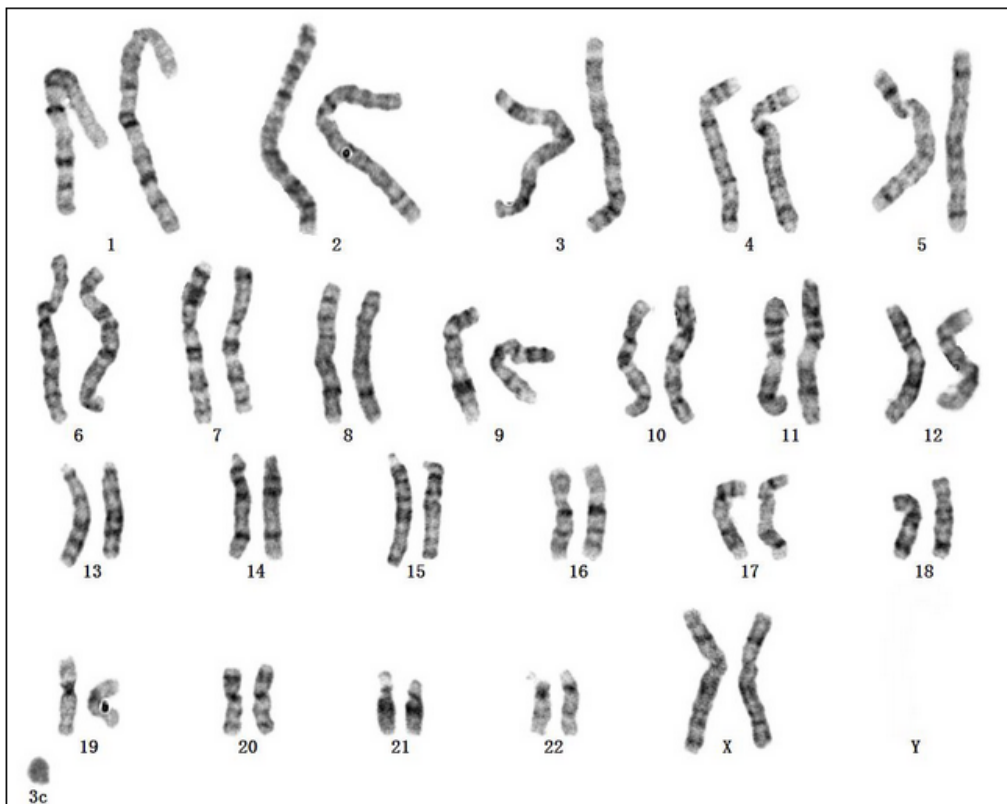
► *What patient fetal info*

Patients : about the genetic t the speci counselin gynecolo to a gene training :

回到Marker染色体的病例

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

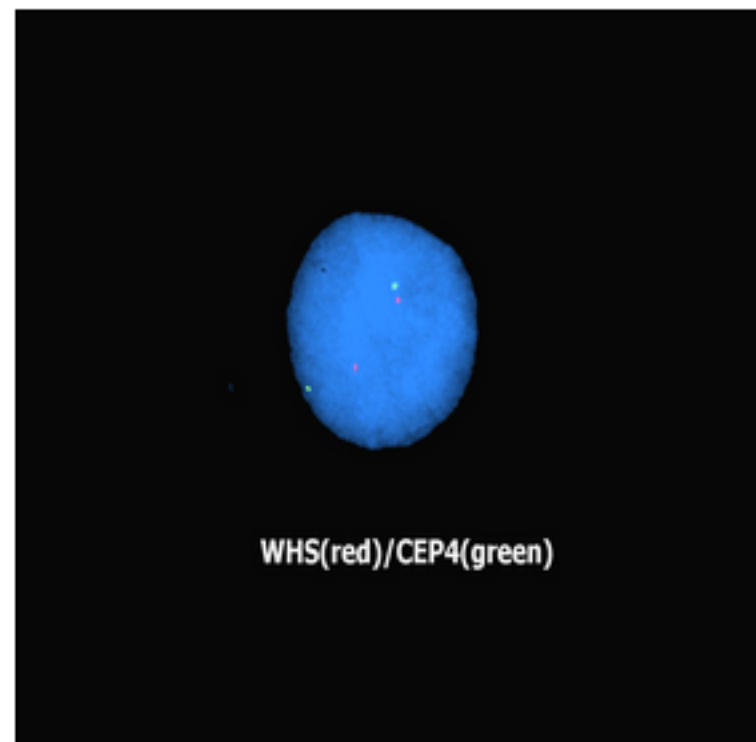
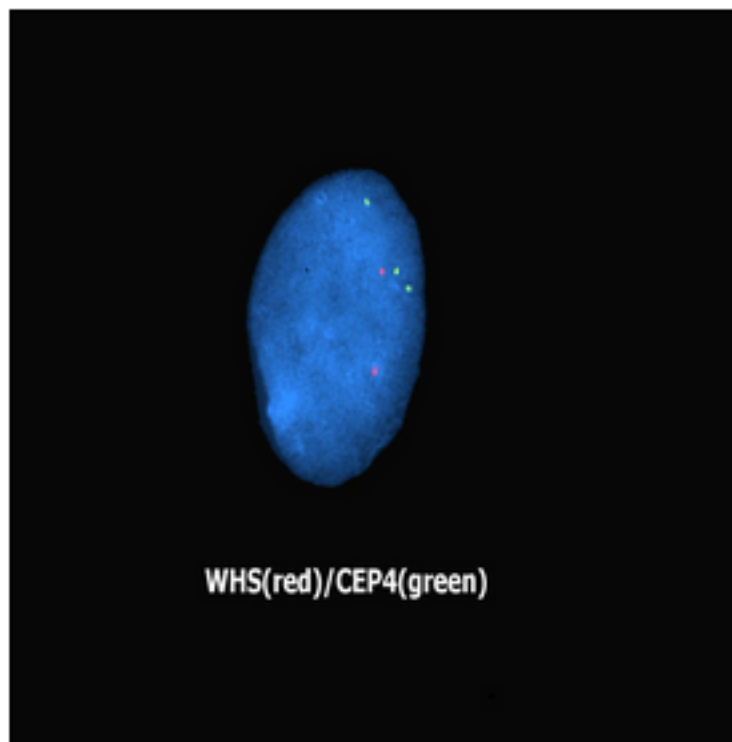
Microarray 是目前可以选择的好方法

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

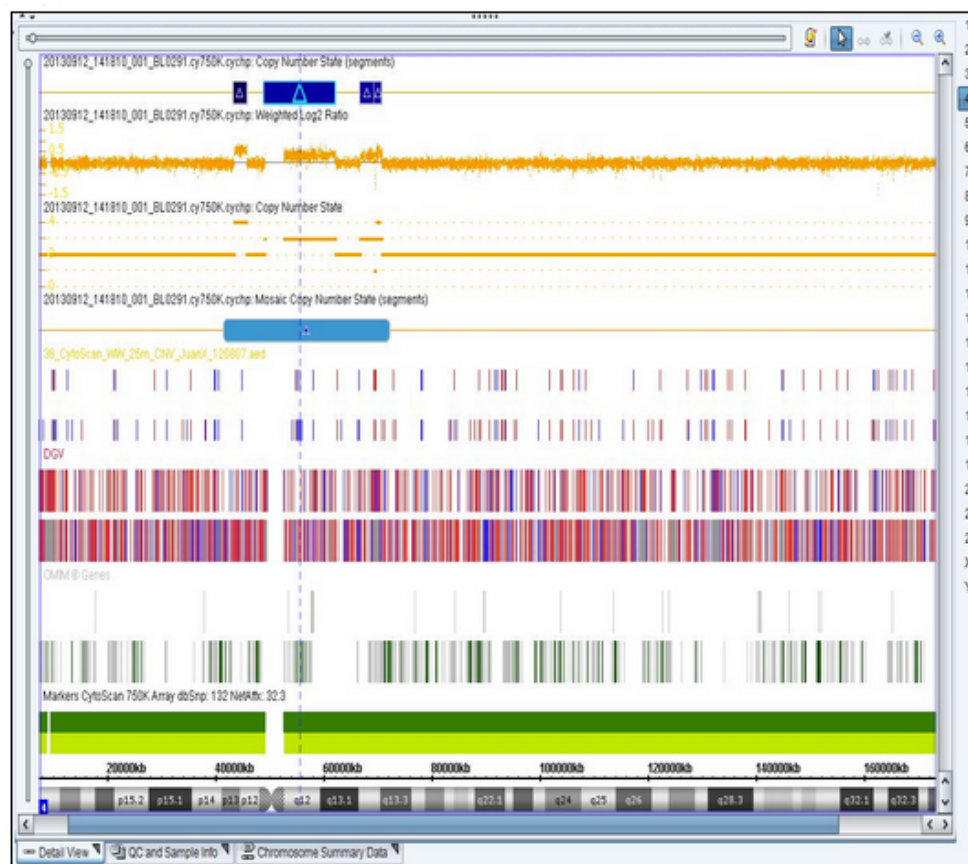
Microarray检测显示胎儿4号染色体4p13q13.3区段有30.537Mb的嵌合性重复



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。

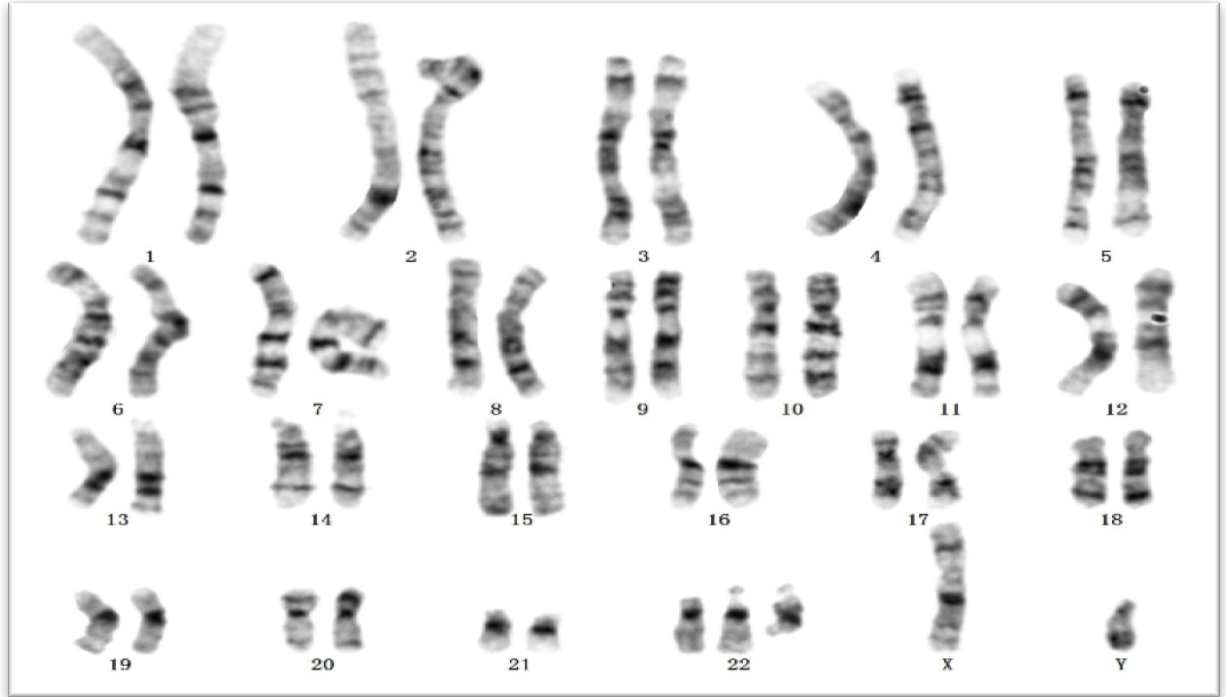


明确诊断后根据Microarray的检测结果进行详细的数据库检索和病例检索：

- 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

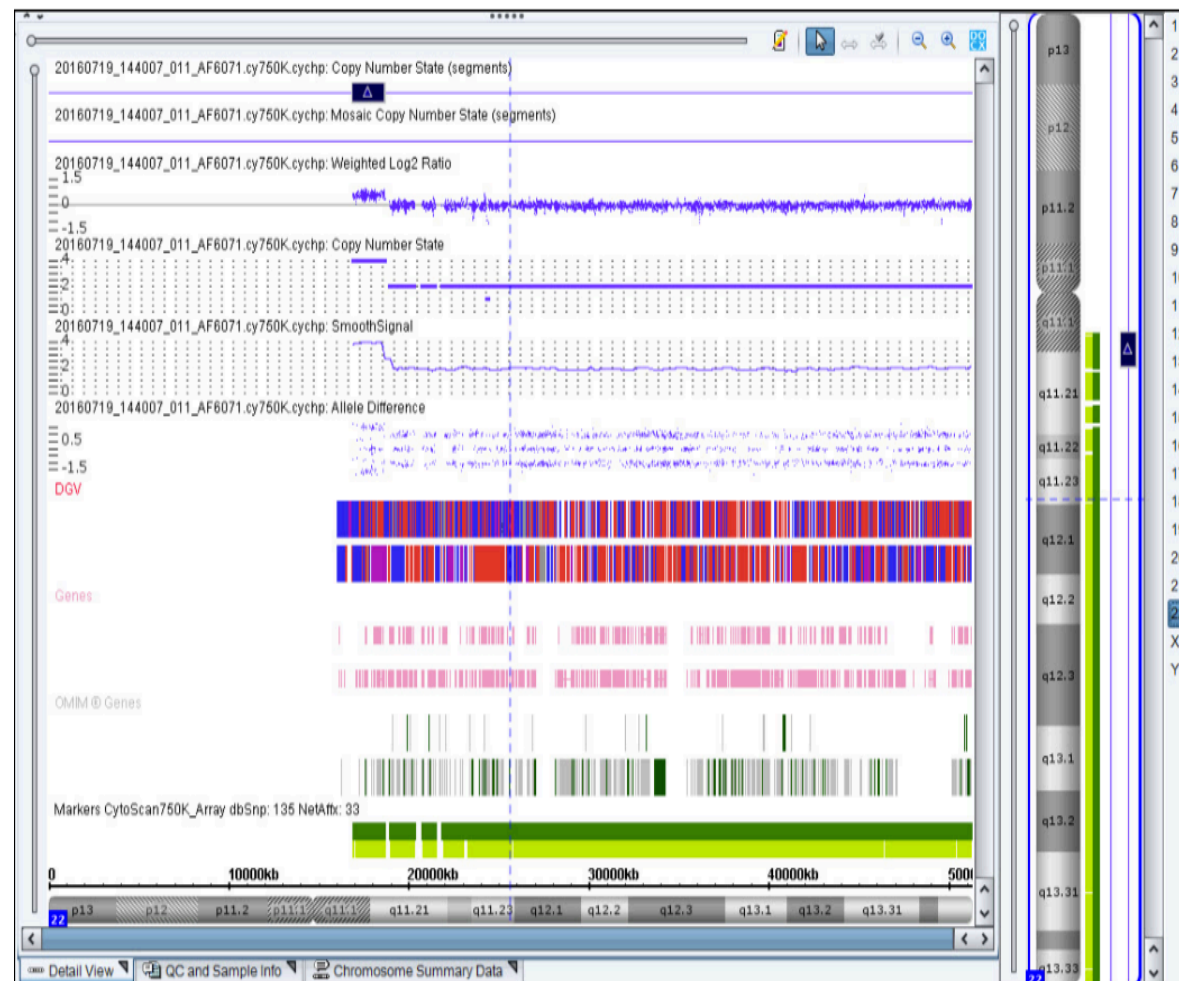


47, XY, +mar, del (22) ?

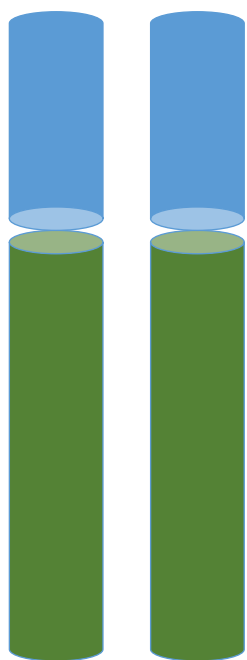
病例-microarray结果

- 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

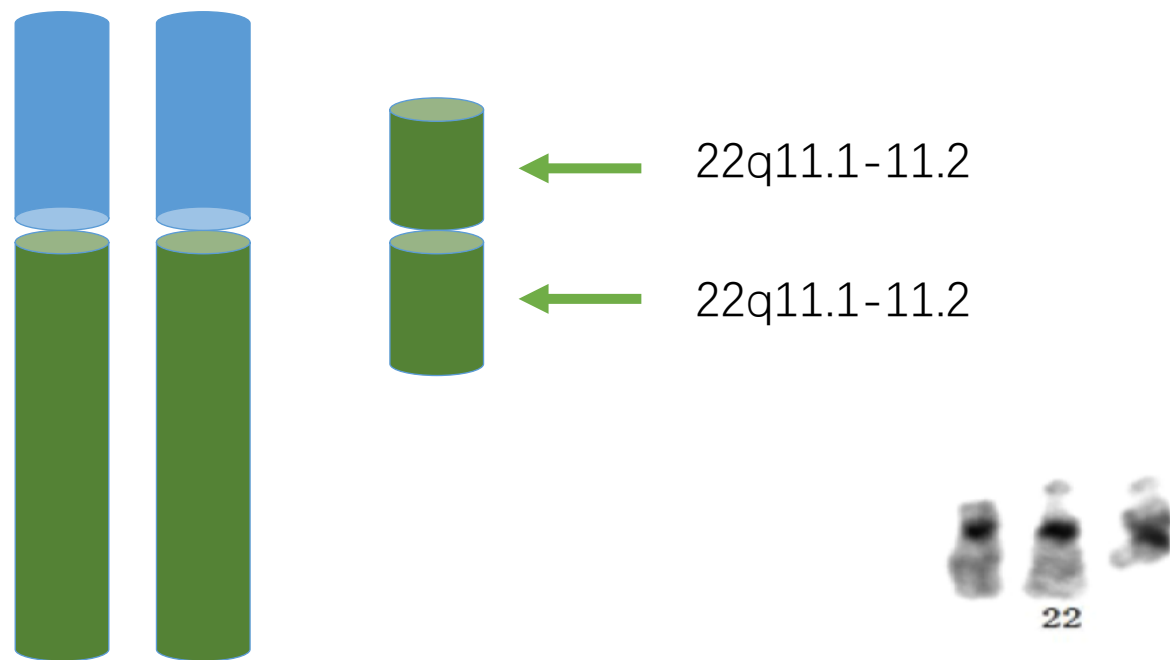
arr[hg19] Yq11.23(26,527,669-27,456,495)x0,
22q11.1q11.21(16,888,899-18,649,190)x4



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/瘻；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

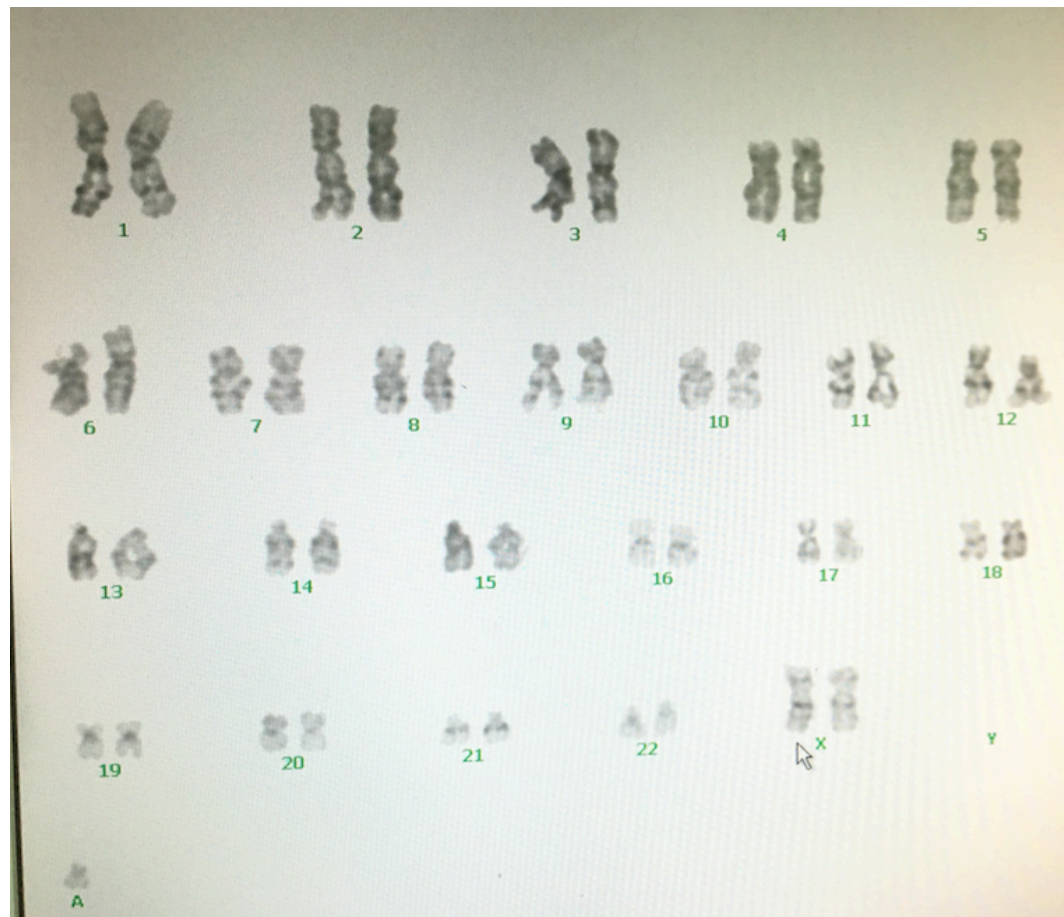


诊断心得

- Microarray是一种强大的染色体分析分子技术，目前已经全面进入临床应用
- Microarray检测是明确产前诊断中遇到的marker染色体性质的有效方法
- 因产前超声异常行介入性产前诊断的病例，应同时进行microarray的检测
- 当NIPT检测有不明确的意外发现时，应行介入性产前诊断加以明确诊断，由于这些病例多为染色体节段性的异常，所以同时进行microarray的检测是必要的

病例

- 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

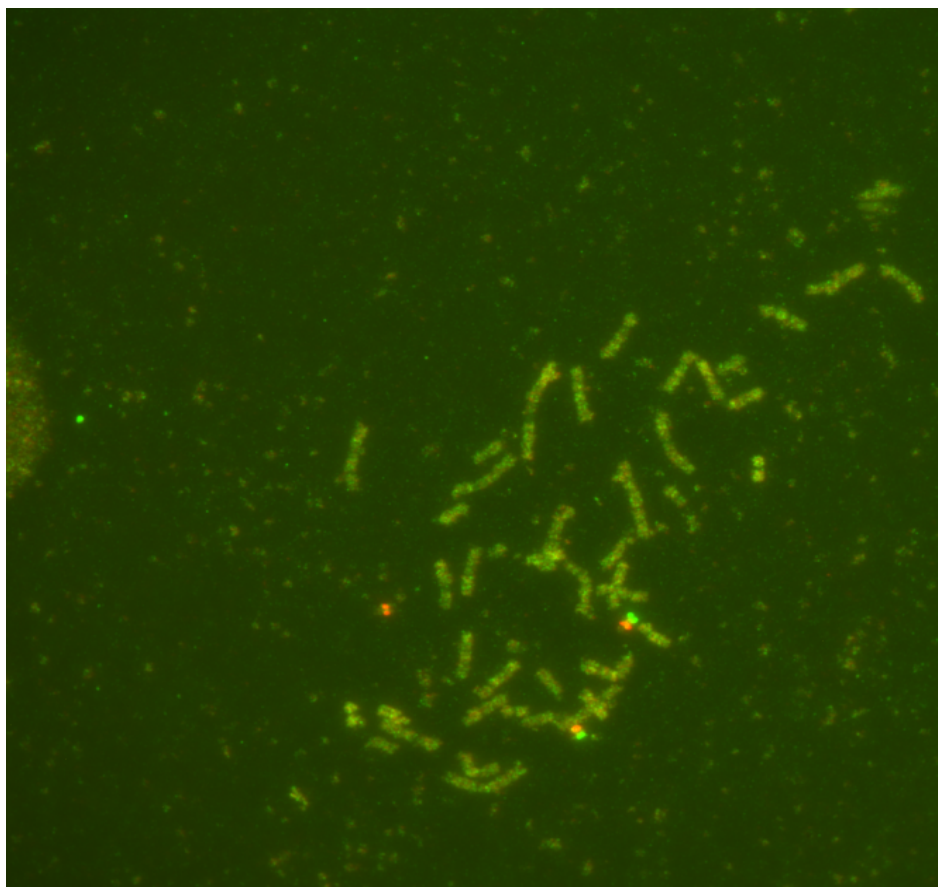
病例：microarray结果

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

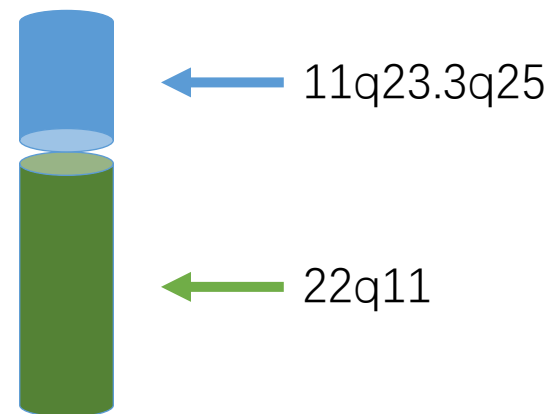


病例：结合FISH诊断结果



GLPTUPLE1 (22q11) 红 /GLPARSA (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

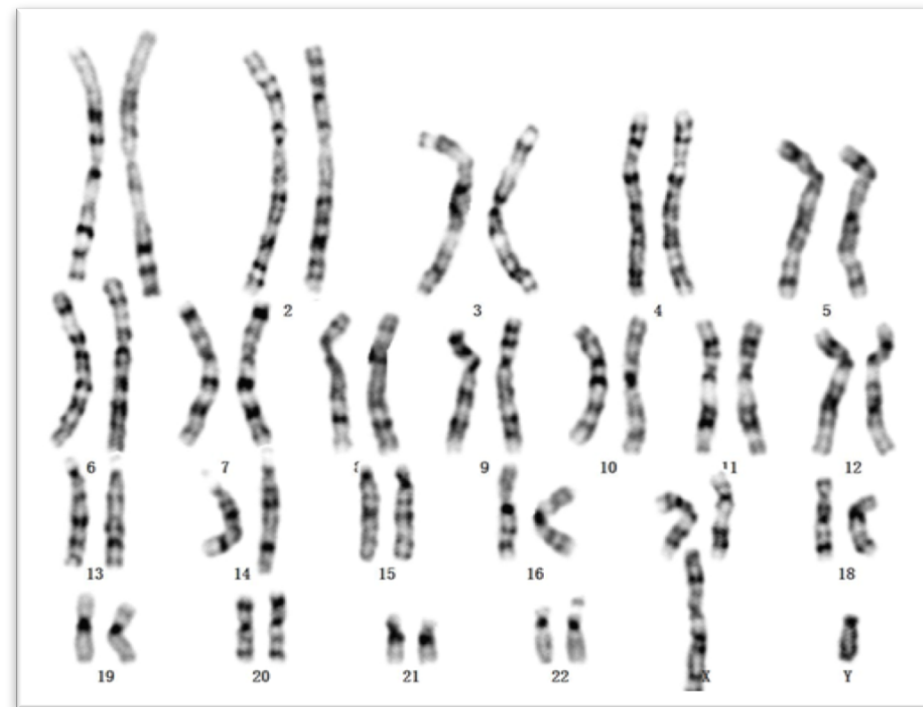


Marker 染色体

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

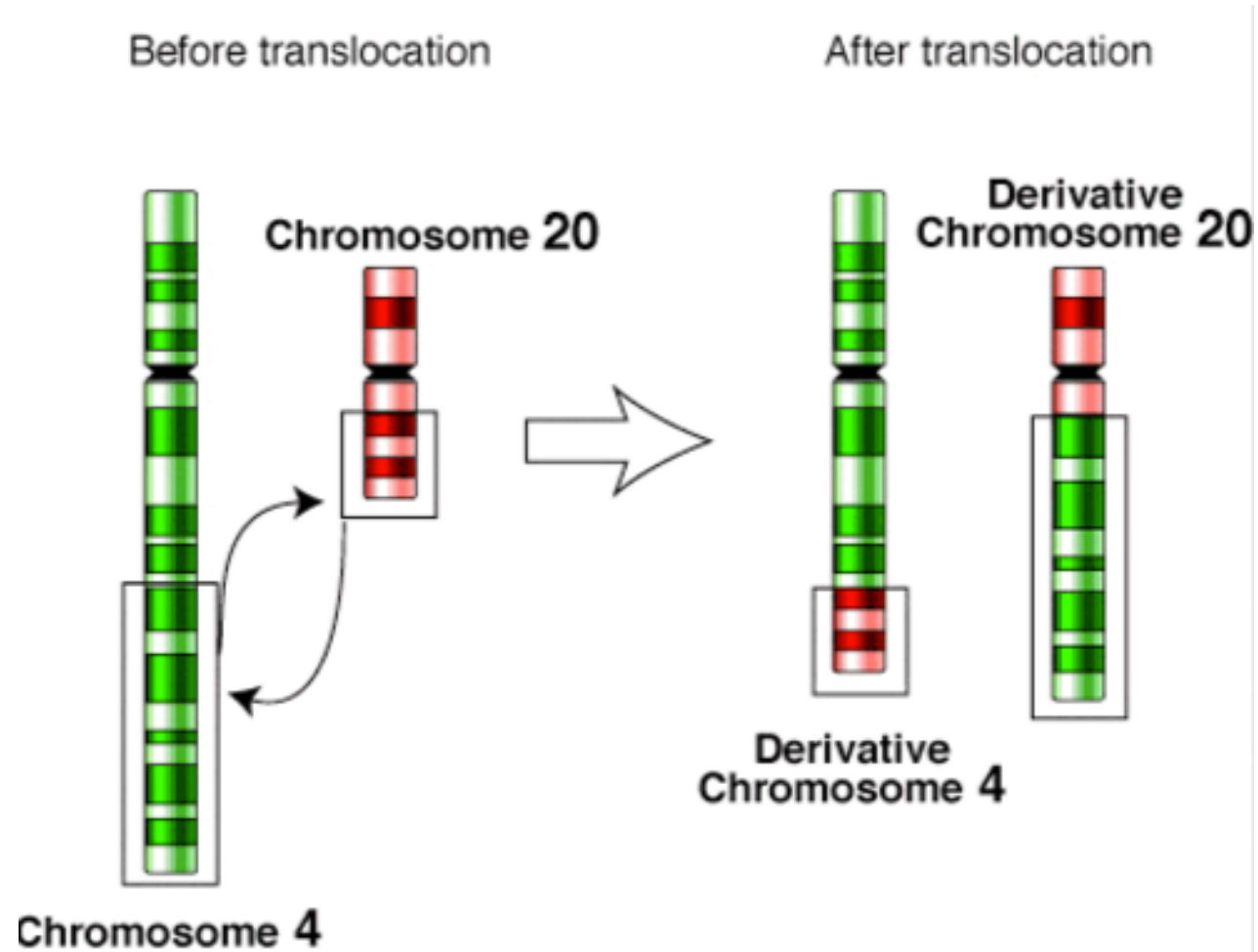


46, XX, t(11;22)(q23.3;q11.2)

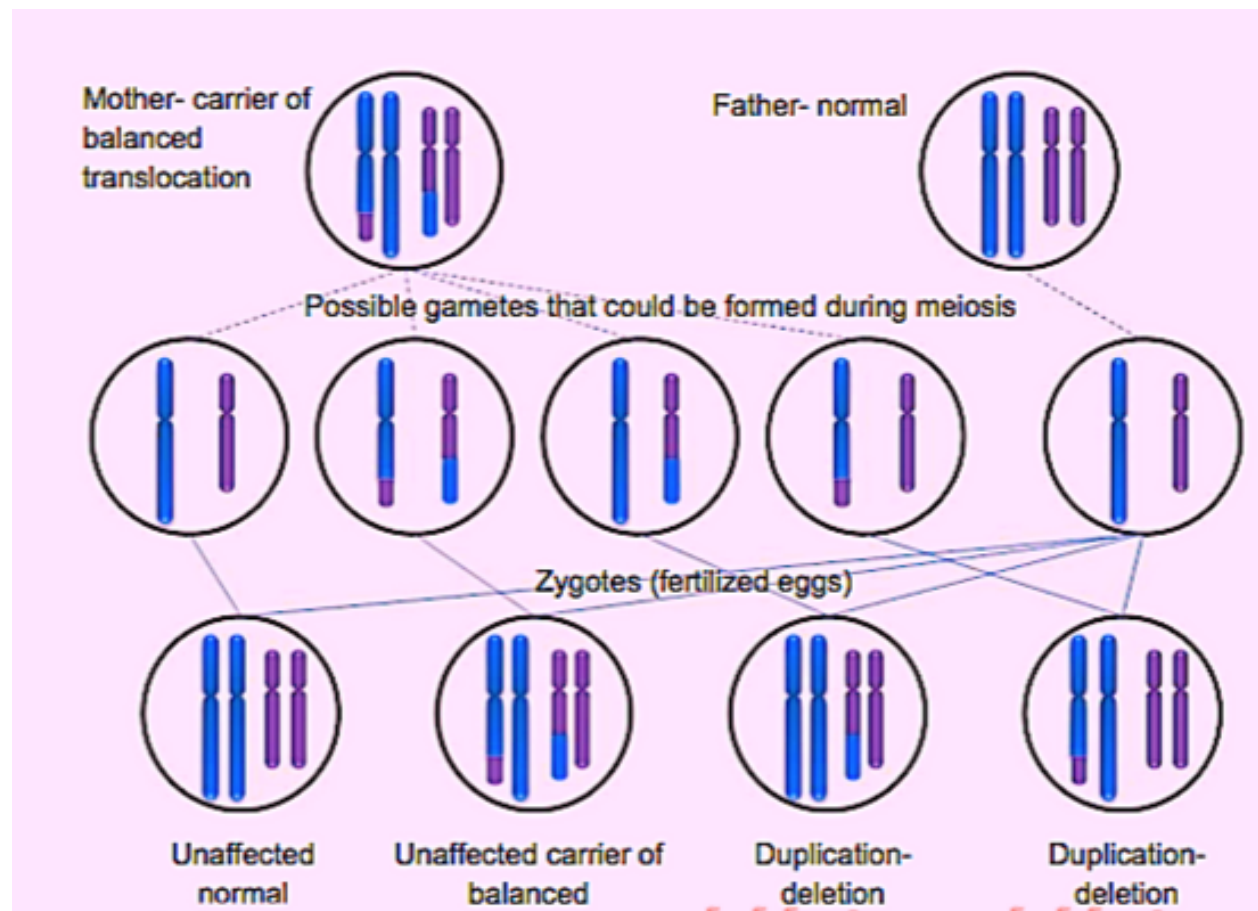


46, XY

染色体平衡易位的发生

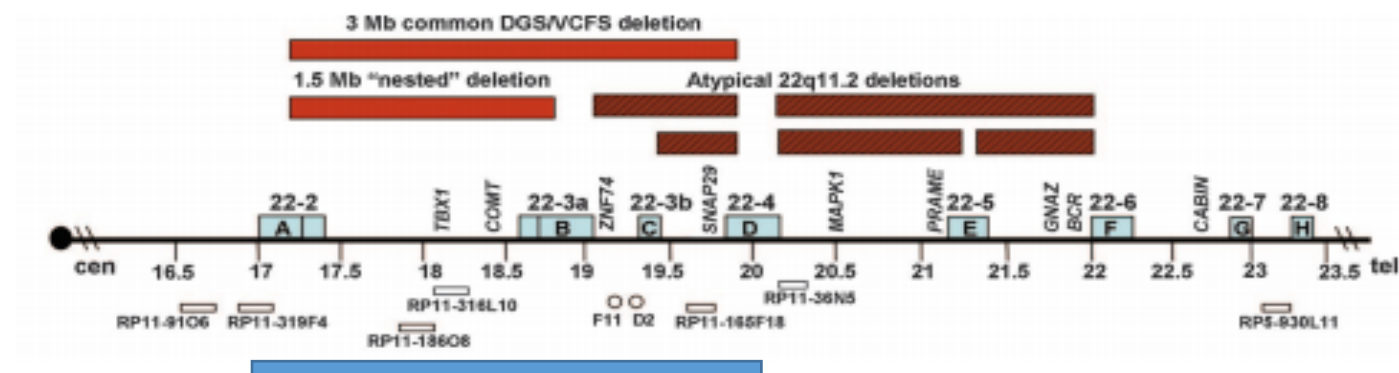
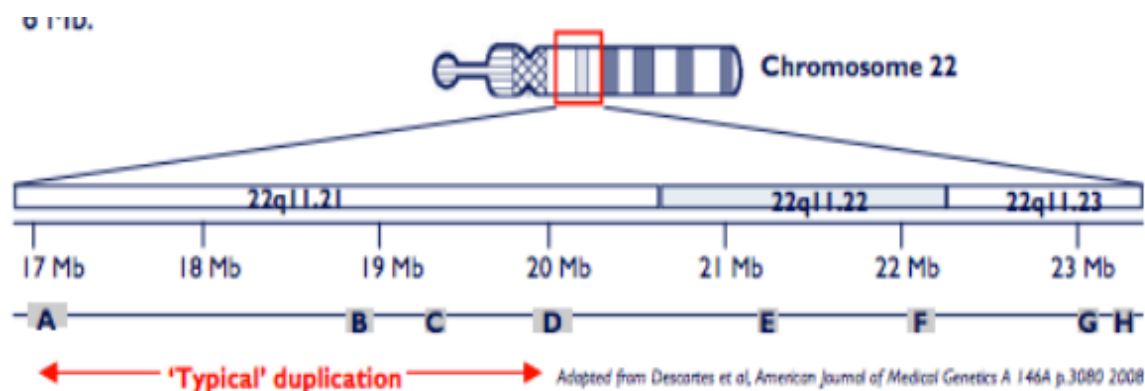


平衡易位的患者后代易出现反复的流产



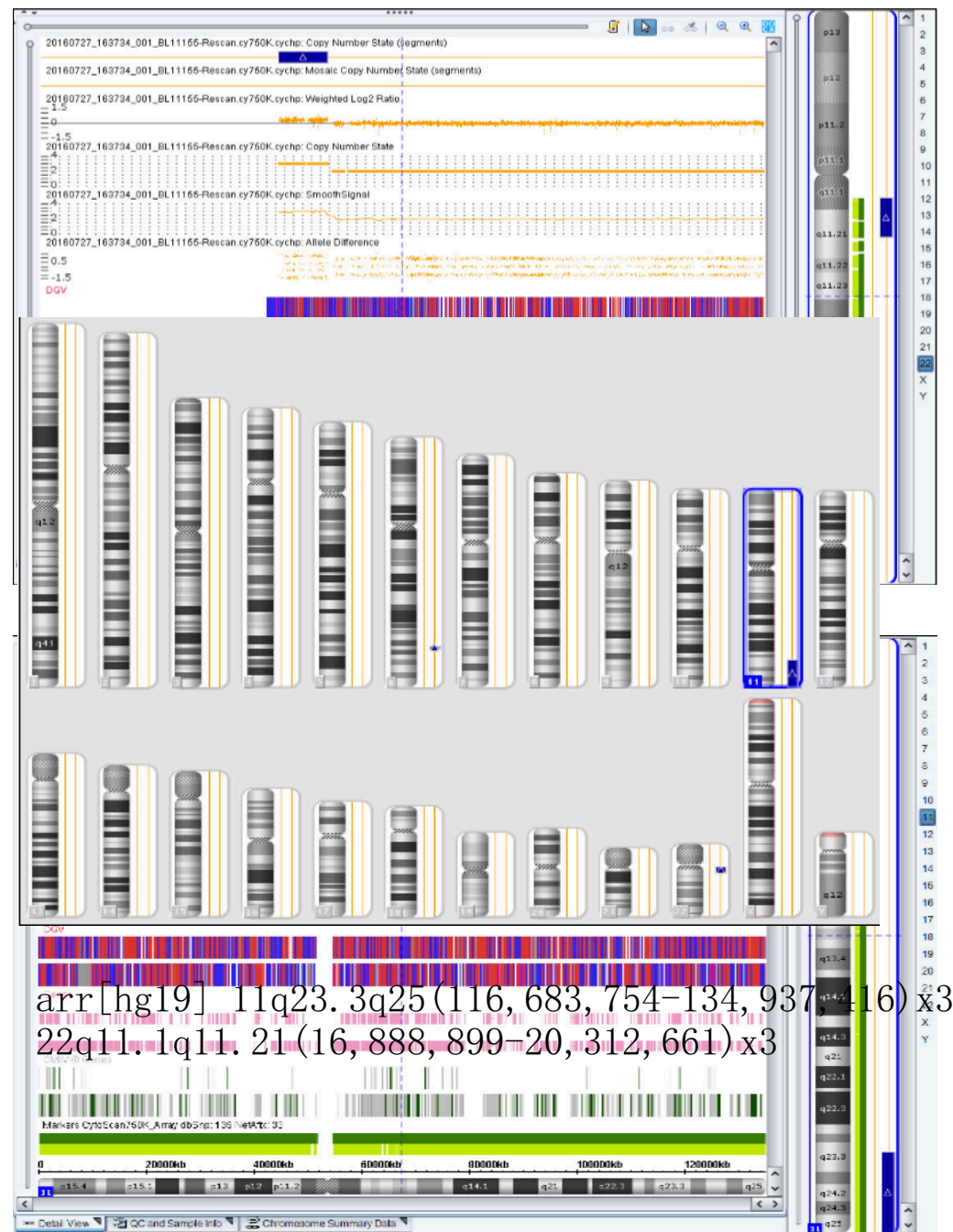
22q11.2微重复综合征

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

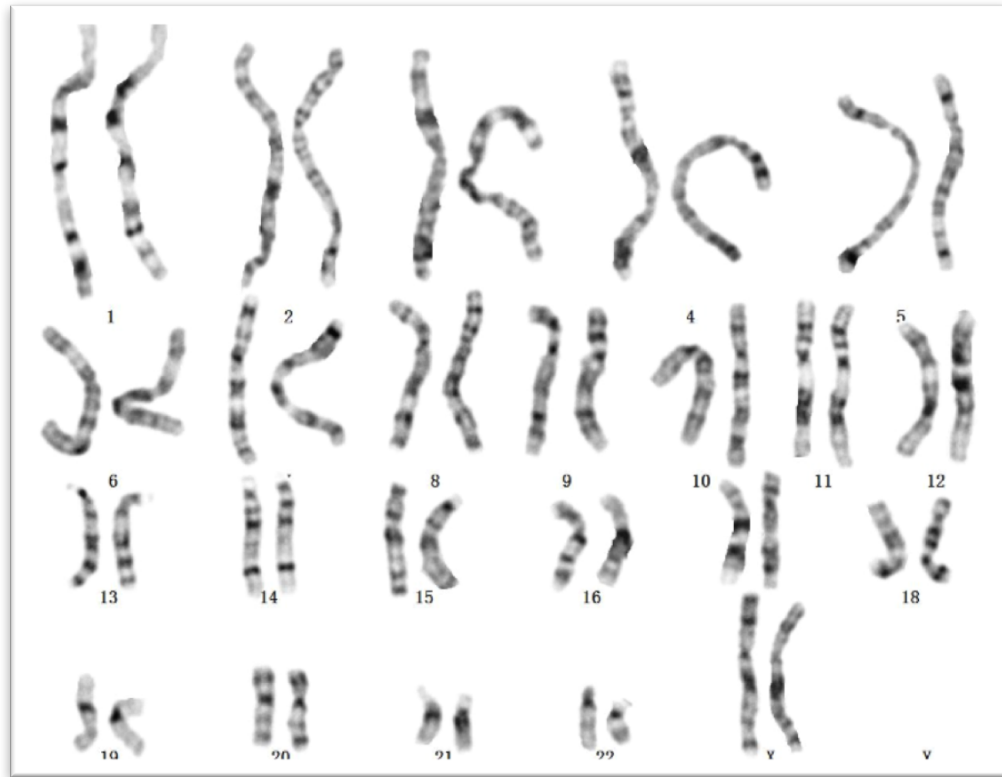


病例：生后的诊断病例

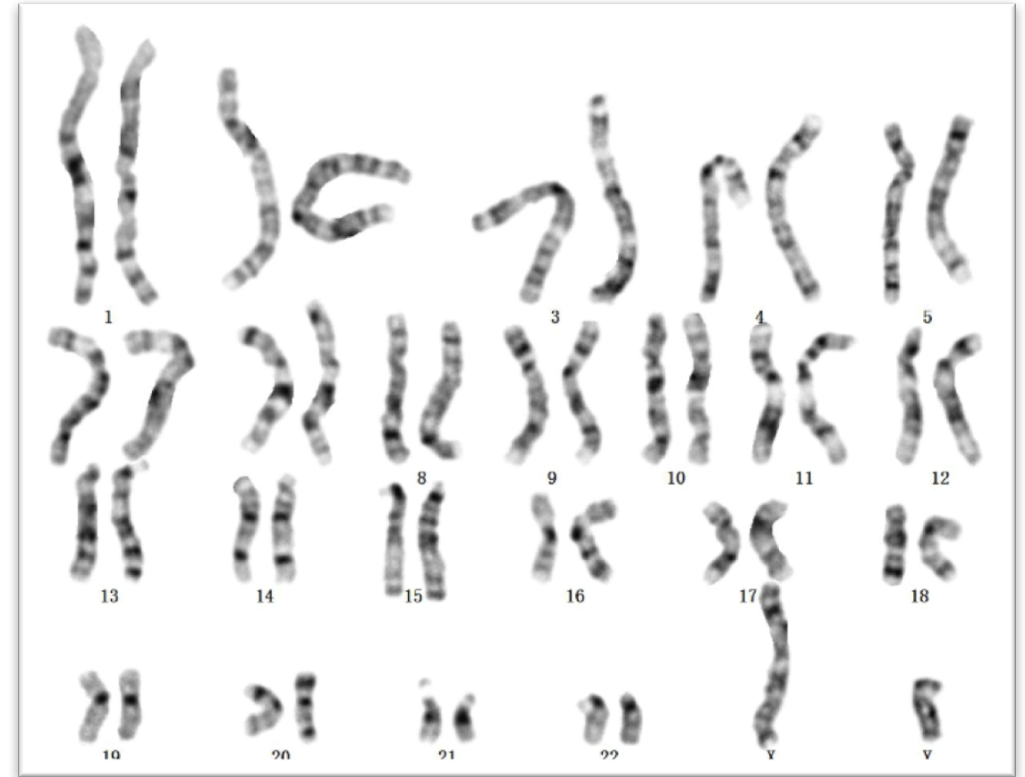
- 3岁男孩，因先天性室缺，体格发育慢，认知及语言能力差就诊
- 外院查外周血核型分析正常
- Microarray芯片检测结果显示患儿11号染色体11q23.3q25区段存在18.2Mb片段的重复，内含KCNJ1, FLI1, JAM3等123个OMIM基因，涉及Jacobsen综合征疾病区域。已有研究报道该片段重复与头面部异常，智力低下，语言发育迟缓等疾病相关。
- 患儿22号染色体22q11.1q11.21区段存在3.4Mb片段重复，内含CLTCL1, HIRA, TBX1等37个OMIM基因，涉及Emanuel综合征 (Emanuel syndrome)、22q11.2微重复综合征 (Chromosome 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。



夫妇外周血染色体分析



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



46, XY

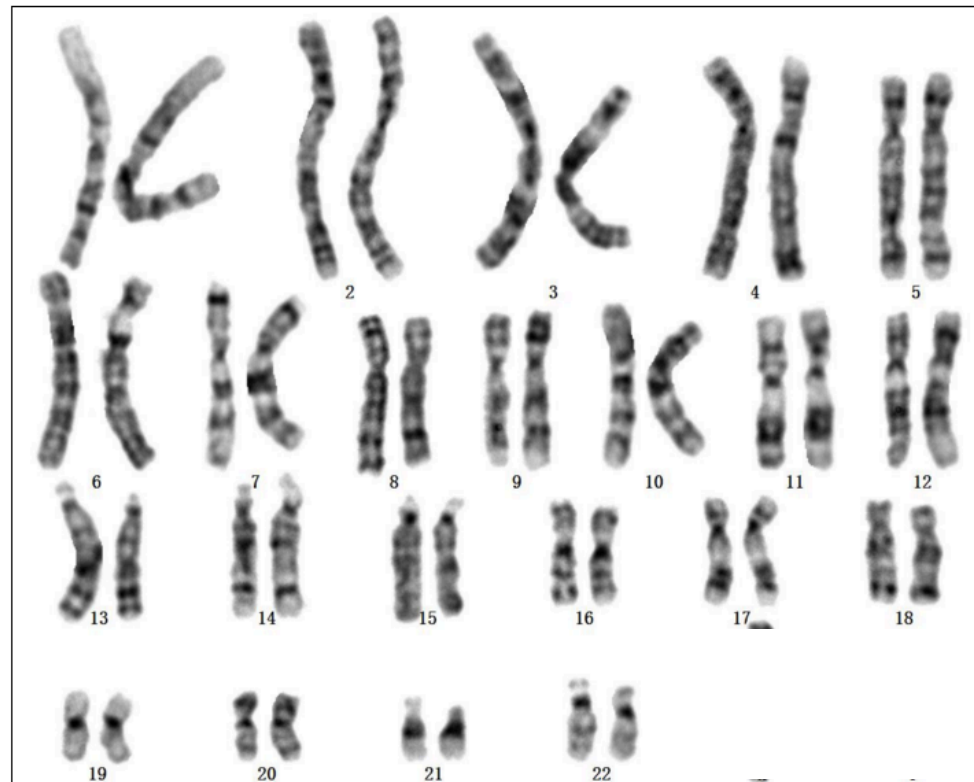
病例诊断心得

- Microarray是产前诊断染色体微缺失微重复异常的重要技术
- 22q11.2区域存在大量微缺失微重复的病例，往往有病理性的改变，不同的表现取决于缺失/重复的性质以及涉及的片段位置
- 所以通过microarray检测明确染色体异常的性质及定位，对于胎儿预后判断及遗传咨询是非常重要的
- 这些染色体微重复综合征的起源大多来源于低频率拷贝数重复（low-copy repeats）的重组，但在中国人群中来源于涉及亲代22号染色体平衡异位的情形也占有很大的比例
- 涉及到多个染色体重复/缺失或重复/重复的改变，需要进一步考虑是否由双亲之一平衡异位而引起，明确双亲的染色体情况，对于该类病例的后续妊娠再发风险评估有非常重要的意义

病例

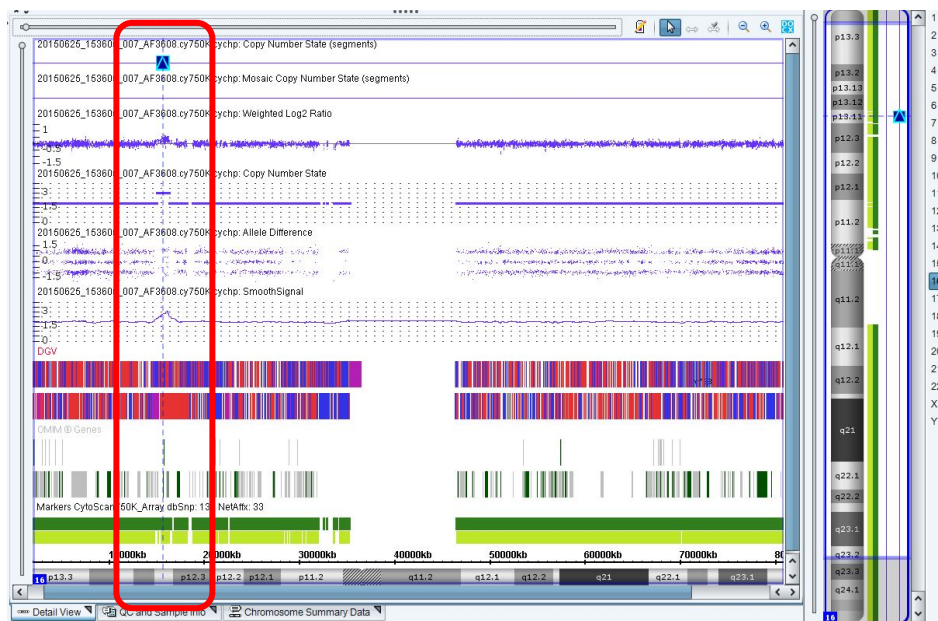
(来自北大人民医院张璘教授提供)

- 33岁，G3P0，既往两次孕中晚期胎儿脑积水引产史
- 12周NT测定 3.2mm/3.8mm
- 15周NIPT检测正常
- 22周起超声反复提示双侧脑室前角囊肿直径3cm
- 25周羊膜腔穿刺，送检核型分析及Microarray
- 核型分析结果正常



46, XN

病例: microarray结果

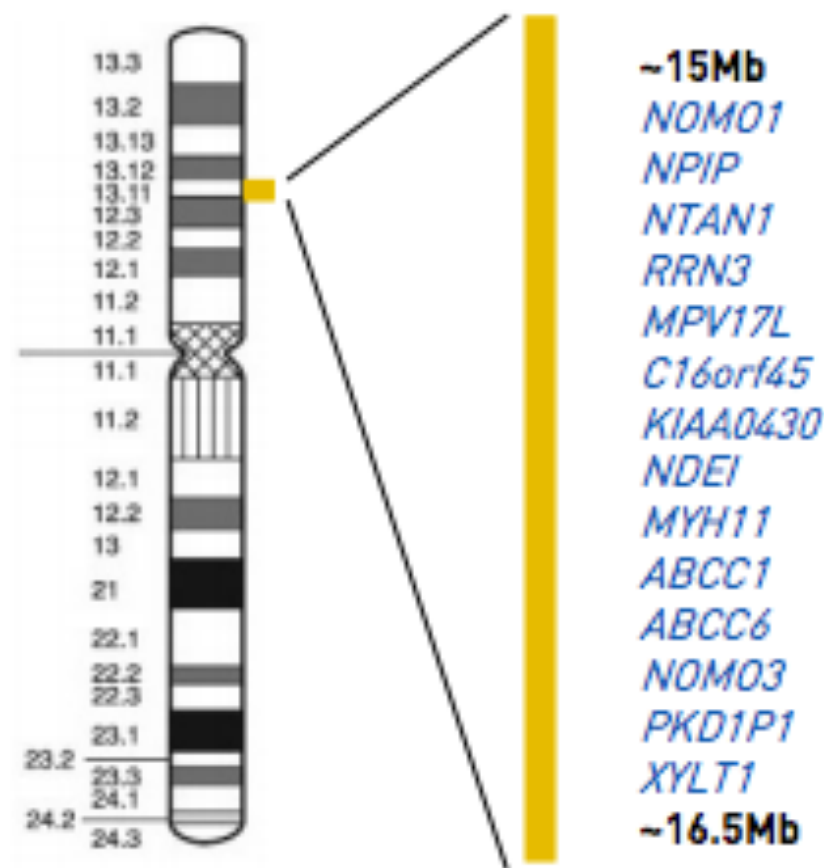


arr[hg19] 16p13.11(15,058,820-16,327,887)x3

Microarray芯片分析显示胎儿16号染色体16p13.11区段存在1.2Mb片段的重复，内含NDE1, MYH11, ABCC6等7个OMIM基因，位于神经认知敏感区域。有研究报告该片段重复与复发性16p13.11重复综合征疾病相关，临床表现包括智力低下，脑发育异常等。但同时也有该片段发生重复而无临床表型的病例报道，其临床意义尚待明确。

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



夫妇外周血核型分析及microarray检测正常



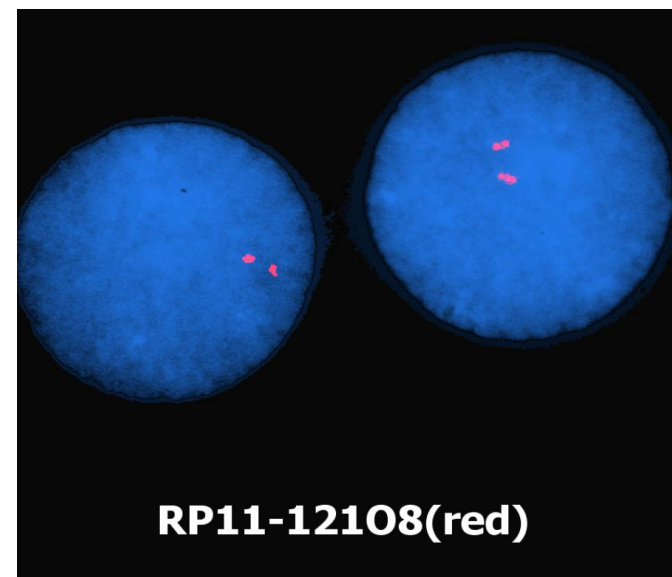
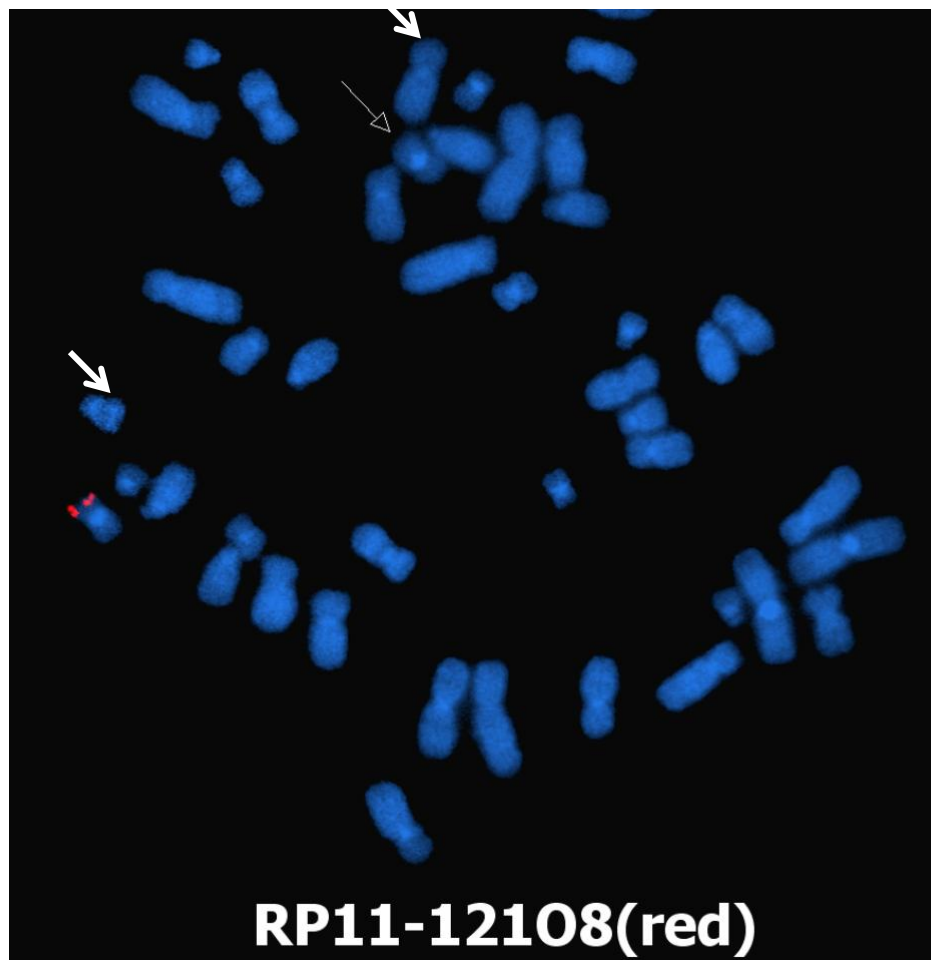
46, XY



46, XX

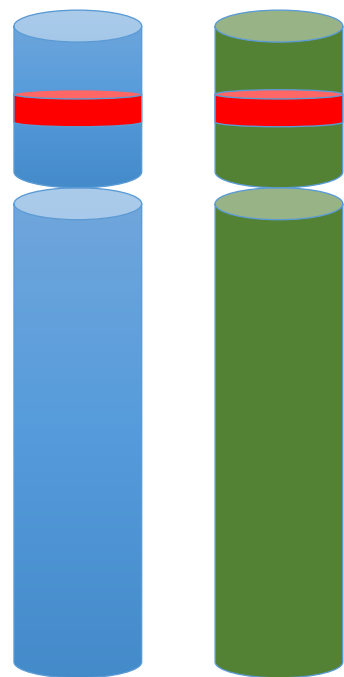
病例：孕妇间期及中期FISH的验证结果

胎儿父母的SNP芯片和高分辨染色体核型分析均显示正常

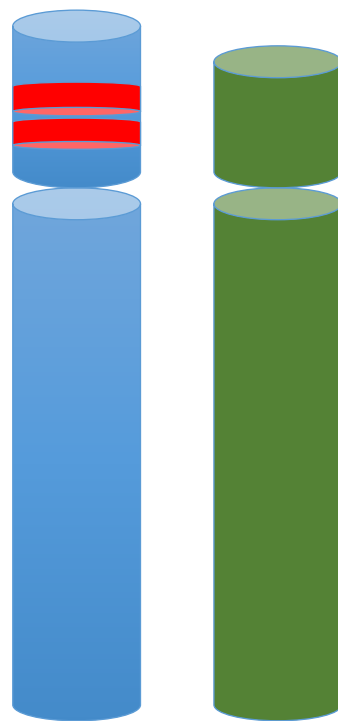


采用位于16p13.11区段的BAC探针
(RP11-12108)的FISH显示：
母亲的一条16号染色体的该片段缺失，
插入到另一同源的16号染色体短臂而
显示此片段的重复。

发生了什么？同源染色体之间的插入



正常的16号染色体



发生插入的16号染色体



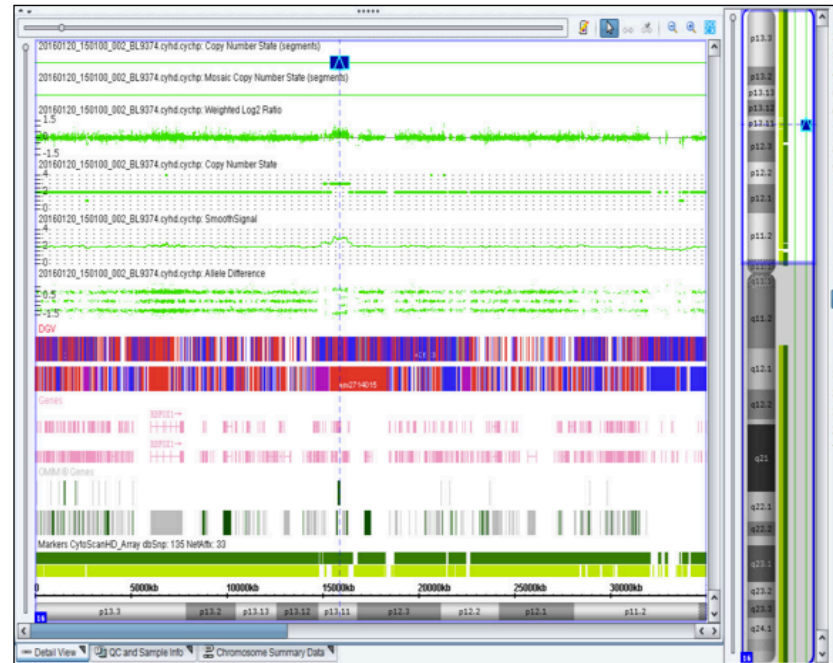
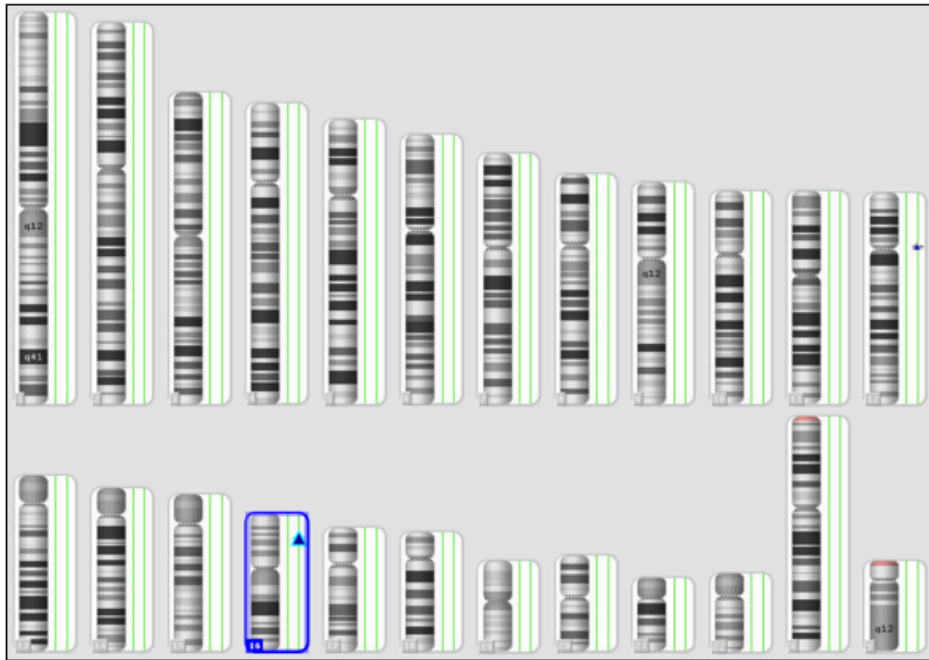
后代配子



该母亲无法
生育该位点
正常的孩子

病例：16P13.11微重复综合征的生后诊断

7岁患儿，疑诊自闭症，伴有学习能力及认知能力的下降

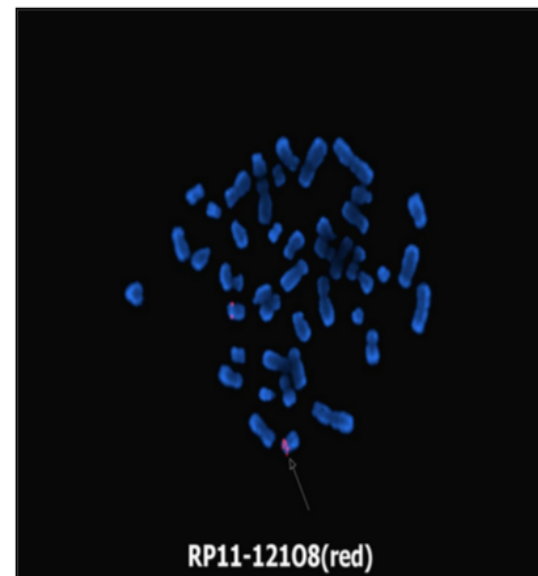
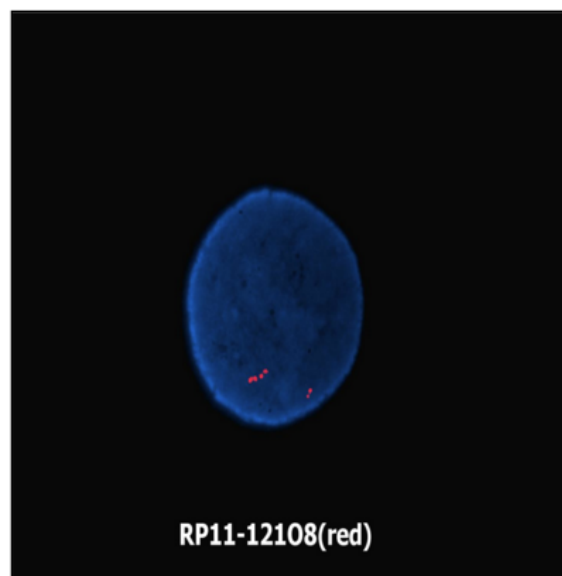


16号染色体16p13.11区段存在965.3Kb片段的重复，内含NDE1, MYH11, ABCC6等6个OMIM基因，该片段位于神经认知敏感区域。

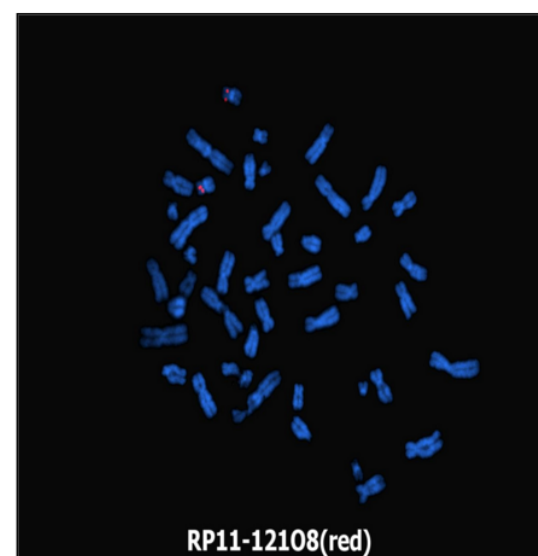
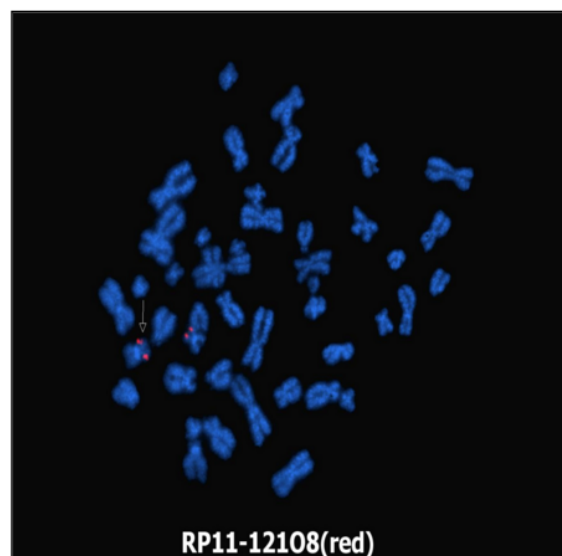
已有研究报道该片段重复与复发性16p13.11重复综合征(16p13.11 recurrent microduplication)疾病相关，临床表型包括智力低下，脑发育异常等

病例：家系FISH验证的结果

患儿：重复



母亲：携带

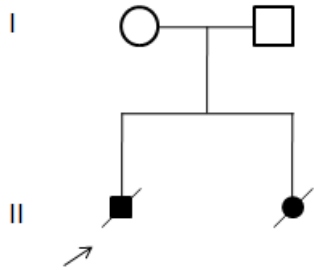


父亲：正常

病例诊断心得 — 小心处理染色体微重复的病例

- 随着microarray检测在产前诊断的应用，临床上经常会遇到产前发现胎儿微缺失微重复综合征的情况
- 有许多微重复综合征患者有异常的表型，但也在正常人群中有一定比例的分布
- 当临床上遇到这类病例时，双亲的验证是非常重要的
- 验证的方法视发生异常改变的染色体片段大小而定，中期FISH或microarray技术是重要的方法
- 对于双亲之一也携带有同样的异常但无表型时，可以降低胎儿病理性诊断的倾向性，但依然不排除以下的可能性：
 - 双亲携带者将来可能发病（成人期发病）
 - 双亲携带者存在表型，但相对较轻，后代的表型可能会比亲代严重
- 针对某一可能致病的微缺失微重复综合征的产前病例，临床医生需要广泛深入学习，了解这类疾病的流行病学特性，以及表型和基因型之间的关系，才能完成高水平的咨询
- 16P13.11微重复综合征发病率较高，临床表型广泛，值得临床重视

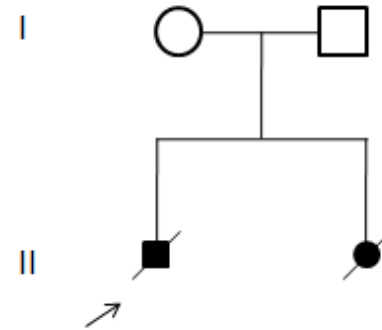
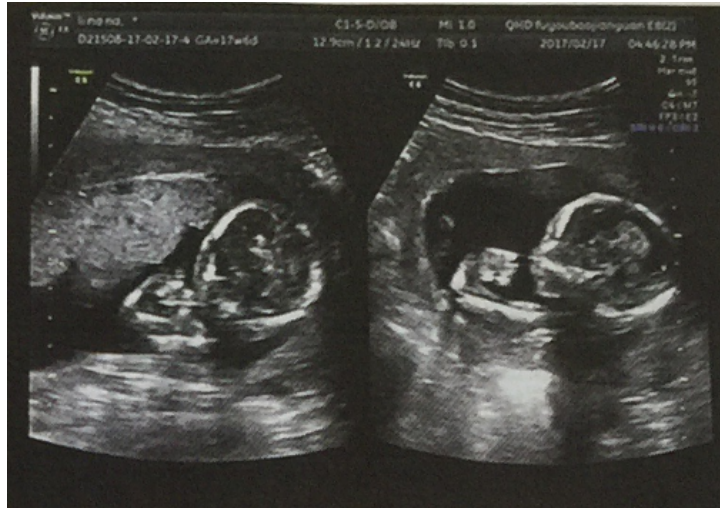
病史 clinical report



- 李某，女，26岁，孕2产0体健。
- 第一胎：
孕12周超声发现双侧前臂一长骨缺失，孕16周超声确诊为双上肢桡骨缺失，孕20周引产一男死胎（外观无明显异常）。未进行遗传检测。

- LNN, a 26-year-old healthy woman
- gravida 2, para 0
- The first pregnancy:
- at 12 weeks of gestation:
the fetus was found of bilateral absence of one of the bones in the forearm by ultrasound;
- at 16 weeks of gestation: bilateral absence of radius was confirmed by ultrasound;
- the pregnancy was terminated without any genetic testing.

病史 clinical report



- 第二胎:
- 孕16周发现双上肢桡骨缺失;
- 孕17周羊穿, SNP array检测。

- The second pregnancy:
- at 16 weeks of gestation:
the fetus was confirmed of bilateral absence of radius by ultrasound exam;
- Amniocentesis at 17 weeks of gestation; SNP array was performed.

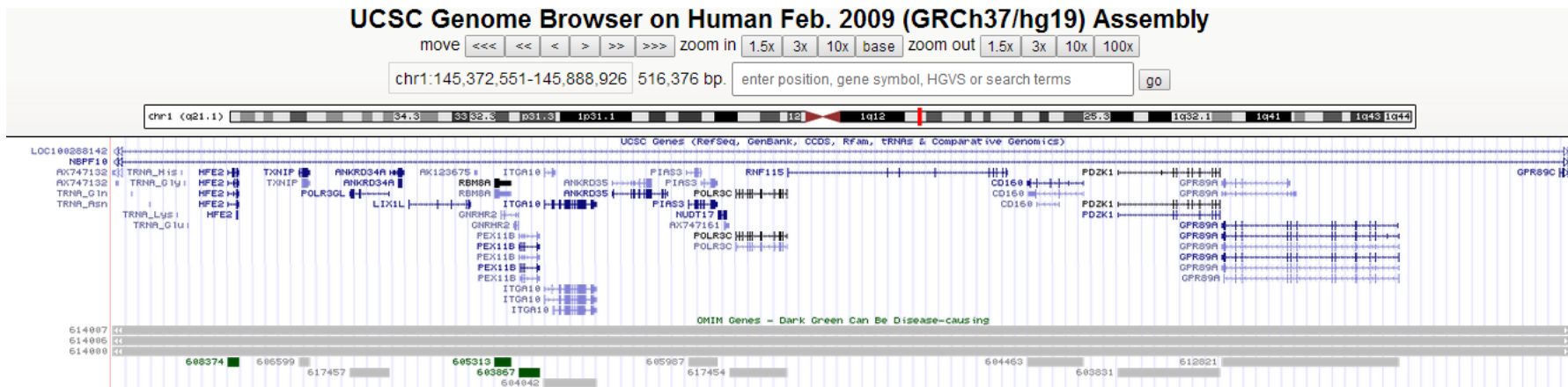
CMA及核型分析检测结果

羊水核型：46，XN

CMA: arr[hg19] 1q21.1(145,372,551-145,888,926)x1



检测结果 RESULTS



● 解读：

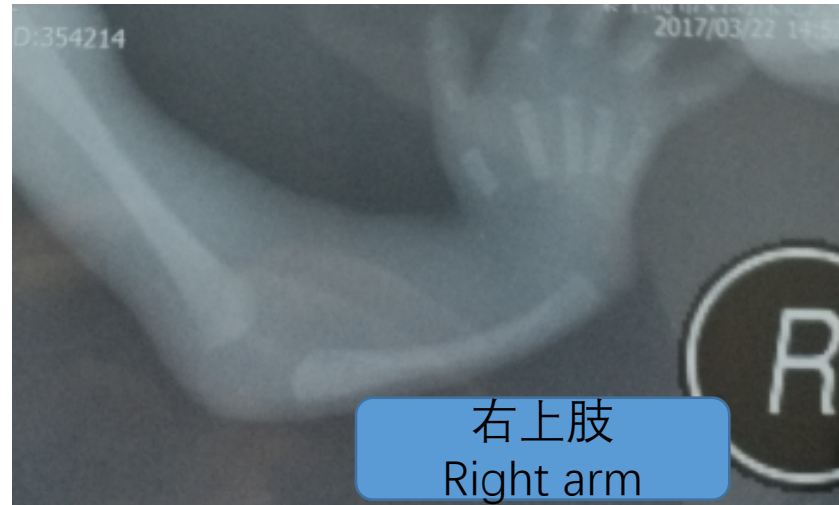
- 内含RBM8A等10个OMIM基因；
- RBM8A基因突变/缺失与常染色体隐性遗传的血小板减少伴桡骨缺失综合征相关；
- 已有小于该片段缺失与血小板减少，身材矮小，肢体形态异常，双侧桡骨发育不全，髓鞘空洞症等临床表型相关的病例报道。但也有该片段缺失而无临床表型的病例报道。该片段缺失的临床意义尚不明确。

Interpretation :

- 10 OMIM genes in the deleted region, including RBM8A
- RBM8A, associated with TAR syndrome (thromocytopenia-absent of radius)
- Absence/Dysplasia of radius is common in TAR
- Pathogenic CNV is still uncertain.

X rays of fetus

- 引产 (termination of second pregnancy)
- 唇裂 cleft lip
- X rays : bilateral absence of radius with valgus wrists 双侧桡骨缺失



检测结果RESULTS

1q21.1缺失区间进行高通量测序检测

NGS of the 3 genes related with deleted region of 1q21.1

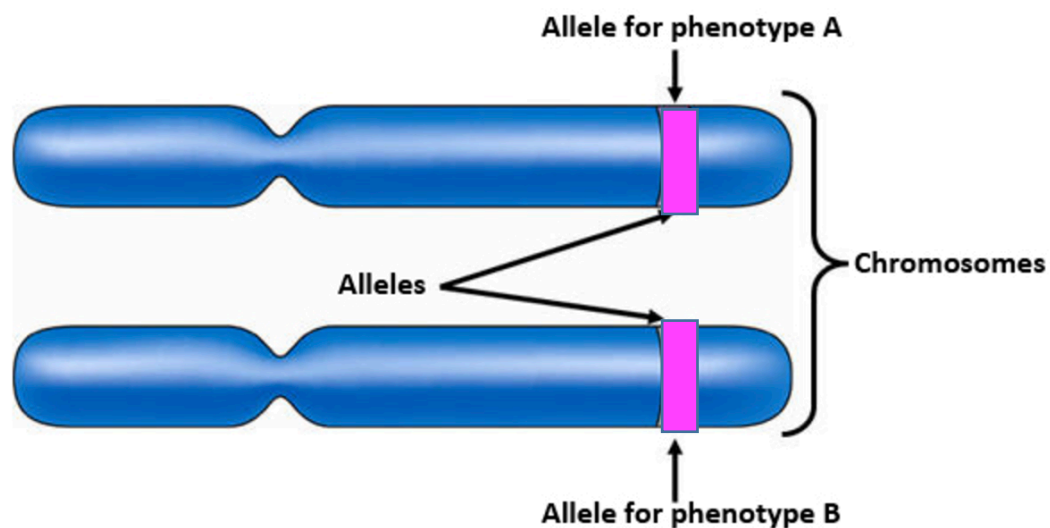
检测结果质控统计：这个综合征基因包检测区间包括 3 个相关基因，13 个编码区总共含有 2,586 个碱基。

平均覆盖深度 77+/-26X，大于 10X 覆盖区间占 100%，大于 20X 覆盖区间占 100%。

| 基因名称 | OMIM 编号 | 遗传 方式 | HG19 位置 | 转录本 | 核苷酸与 氨基酸改变 | 合子 状态 | 人群 频率 | ACMGG 变异分类 | 相关疾病/文献 | 来源 |
|-----------------------|------------|----------|----------------------------------|-----|---------------------|-----------|----------|---------------|-------------------|--------------------|
| 1q21.1 缺 失 | - | AR | chr1: 145414782- 145522919 | - | 108KB 缺失 | 杂合 | - | II 类- 可能致病 | 血小板减少伴桡 骨缺失综合征 | 父亲 (杂合) |

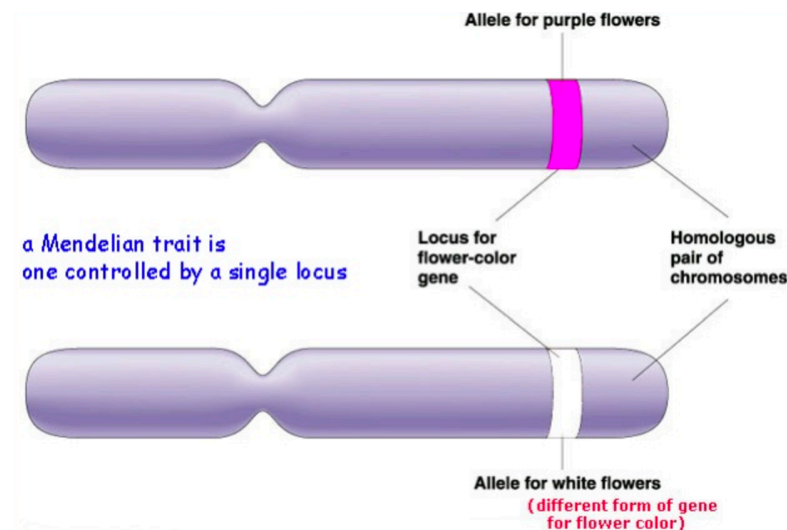
- 芯片及高通量测序均检测到这个片段的缺失遗传自父亲
- 但父亲的表型均是正常的
- 胎儿的仅有的一个缺失不能解释发病的原因
- 但推测一定与该片段中的RBM8A有关
- 母亲本人的RBM8A有什么问题吗？

隐性遗传病的多种可能发病机制



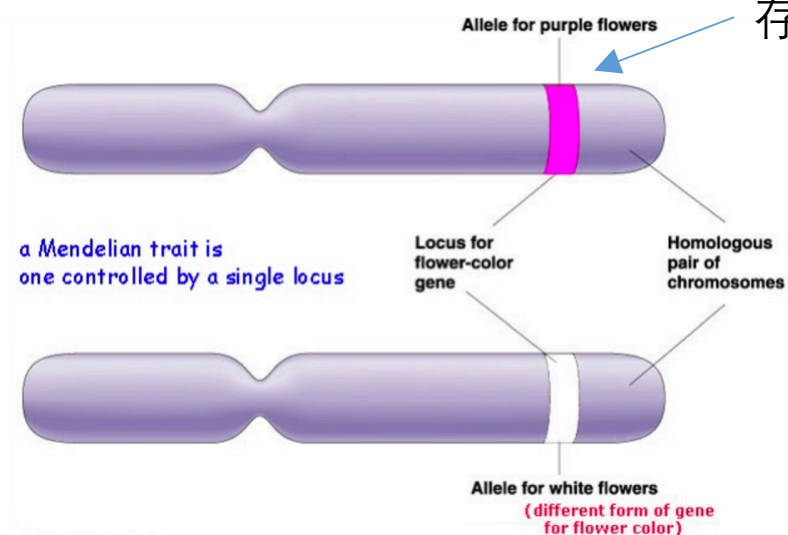
母亲遗传

父亲遗传



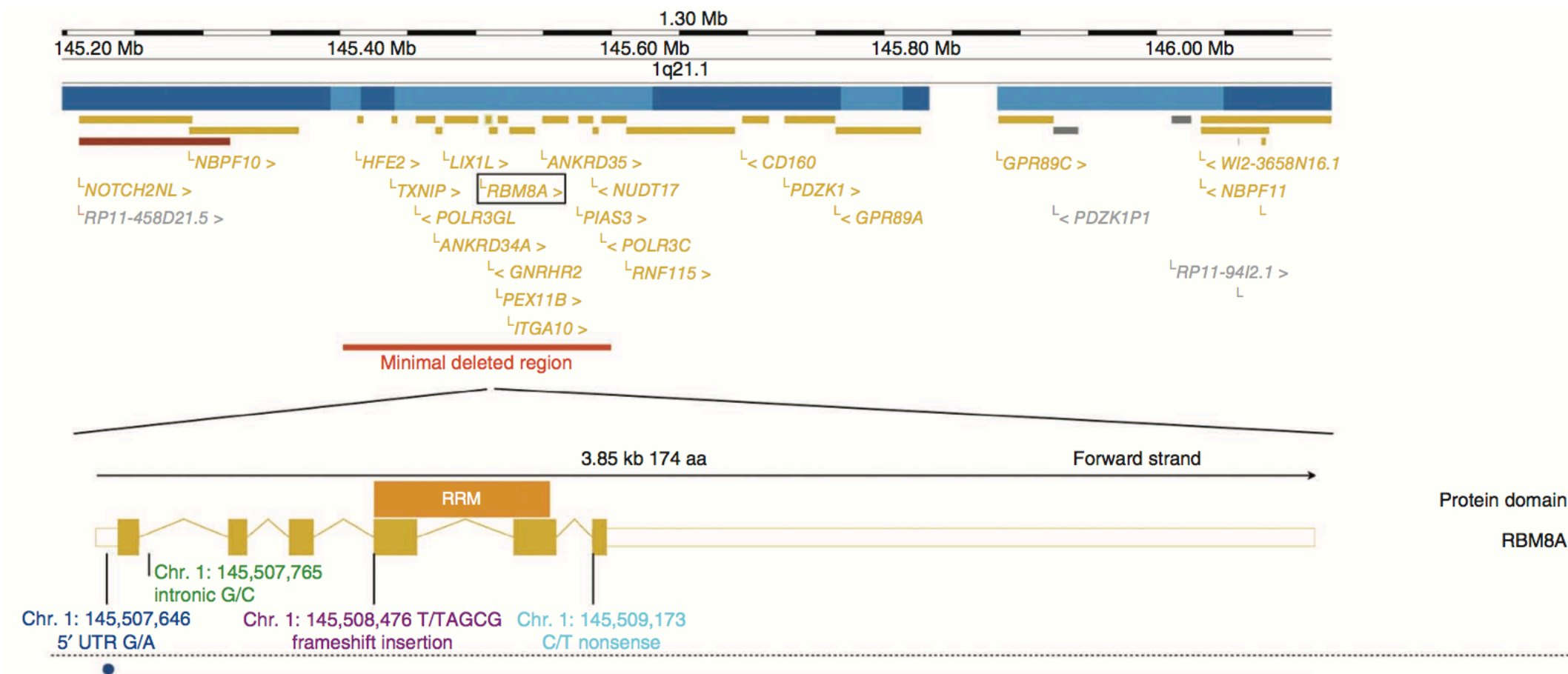
母亲遗传

父亲遗传



存在一个点突变，造成基因的失活

回到RBM8A基因



Compound inheritance of a low-frequency regulatory SNP and a rare null mutation in exon-junction complex subunit *RBM8A* causes TAR syndrome

Cornelis A Albers^{1-3,17}, Dirk S Paul^{3,17}, Harald Schulze^{4,5,17}, Kathleen Freson⁶, Jonathan C Stephens^{1,2}, Peter A Smethurst^{1,2}, Jennifer D Jolley^{1,2}, Ana Cvejic¹⁻³, Myrto Kostadima⁷, Paul Bertone⁷, Martijn H Breuning⁸, Najet Debili⁹, Panos Deloukas³, Rémi Favier⁹, Janine Fiedler^{5,10}, Catherine M Hobbs^{1,2}, Ni Huang³, Matthew E Hurles³, Graham Kiddle^{1,2}, Ingrid Krapels¹¹, Paquita Nurden¹², Claudia A L Ruivenkamp⁸, Jennifer G Sambrook^{1,2}, Kenneth Smith^{13,14}, Derek L Stemple³, Gabriele Strauss¹⁵, Chantal Thys⁶, Chris van Geet^{6,16}, Ruth Newbury-Ecob^{13,14,18}, Willem H Ouwehand^{1-3,18} & Cedric Ghevaert^{1,2,18}

[Albers et al. \(2012\)](#) selected 5 individuals with TAR of European ancestry who had the 1q21.1 deletion and sequenced their exomes, but were unable to find TAR-associated coding mutations in any gene. However, 4 of the cases carried the minor allele of a low-frequency SNP in the 5-prime UTR of the *RBM8A* gene ([rs139428292](#); [605313.0001](#)), while the remaining case carried a previously unknown SNP in the first intron of the same gene ([605313.0002](#)). Genotyping by Sanger sequencing of another 48 cases of European ancestry identified the 2 SNPs in 35 and 11 samples, respectively.

母亲的第一次高通量测序并没有提示该基因有任何突变

Albers (2012) 的一篇文献报道了5例存在1q21.1的缺失，但等位基因找不到任何编码突变的TAR患者，发现其中4例存在*RBM8A*基因5' UTR非编码区的低频率SNP的情况，另一例是在该基因第一个内含子区域存在一个此前未被报道过的SNP位点改变。这两种改变通过Sanger测序可以在其他48例TAR患者中分别检出35例和11例

本例病例是否会存在类似情况？

答案也是否定的

下一步 next setp

- 家系的全外显子测序

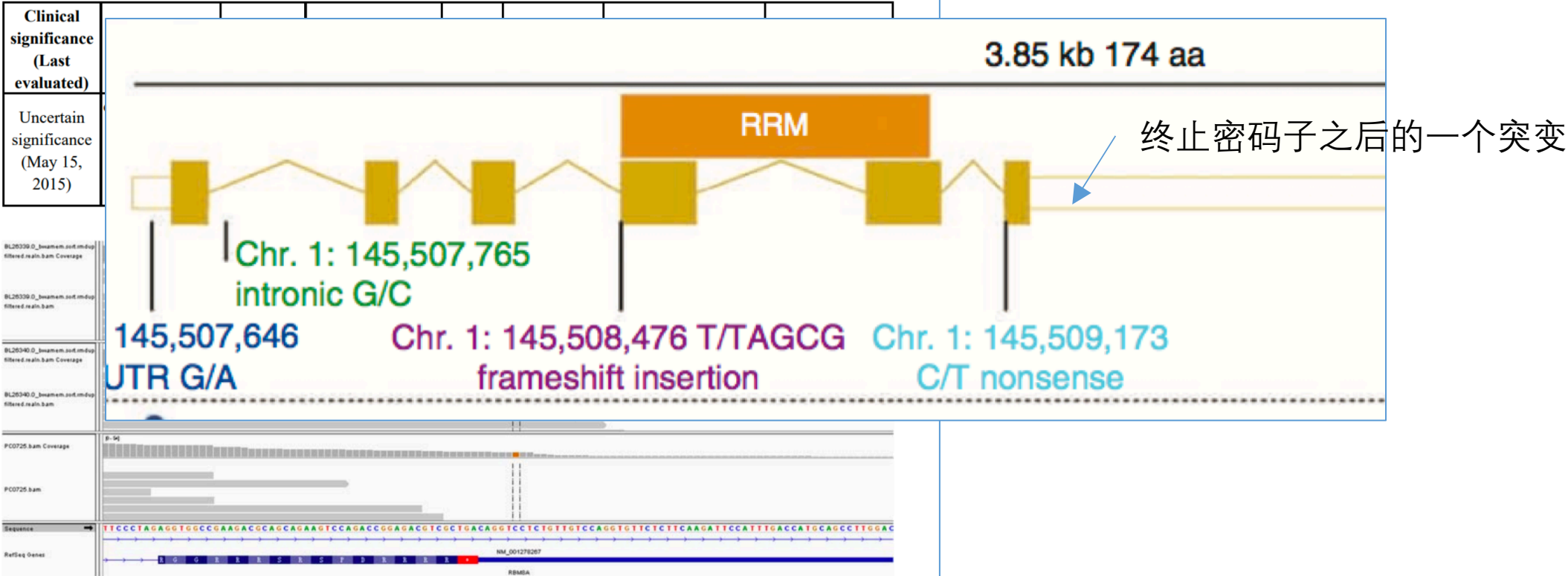
Whole Exon Sequencing for Trio

- 孕妇及胎儿组织的RBM8A基因sanger测序

Sanger sequencing for RBM8A of fetus tissue and mother

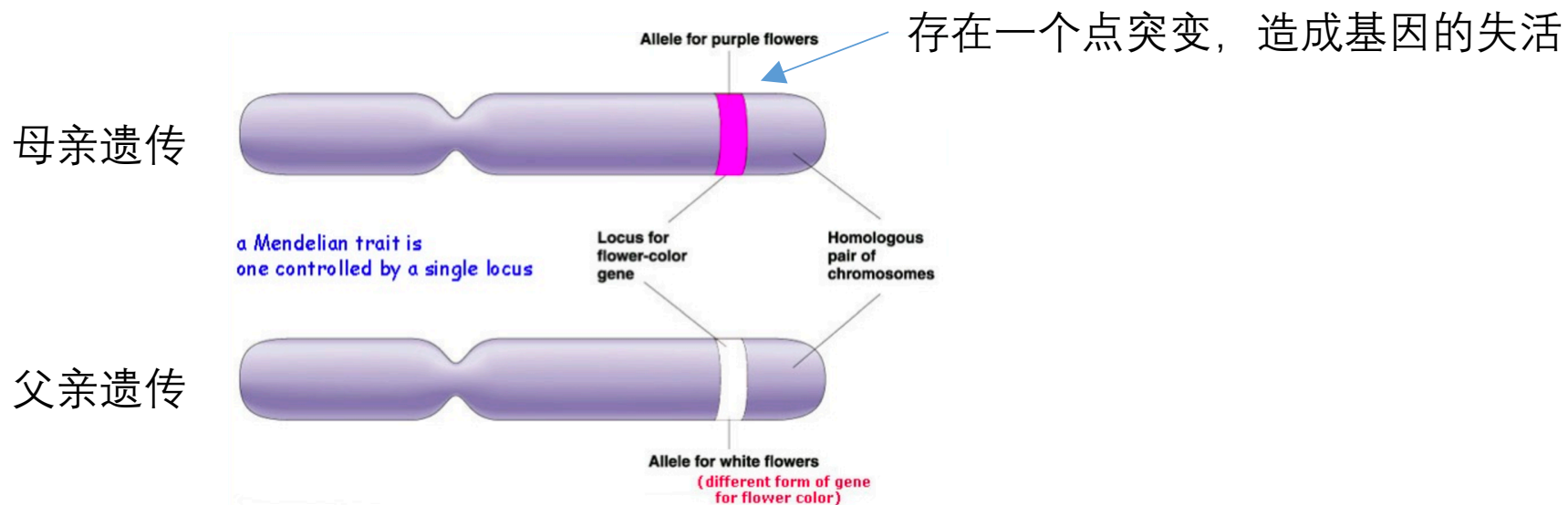
第二次外显子测序和sanger测序终于有了发现

4、RBM8A 第六个外显子发现存在 NM_005105.4(RBM8A):c.*6C>G 变异 (GRCh37: Chr1:145509217, dbSNP: rs12079762), 数据库 ClinVar 注释为临床意义不明。在 BL26339 为野生型, BL26340 为杂合型和 PC0725 为变异纯合型 (如下图)。



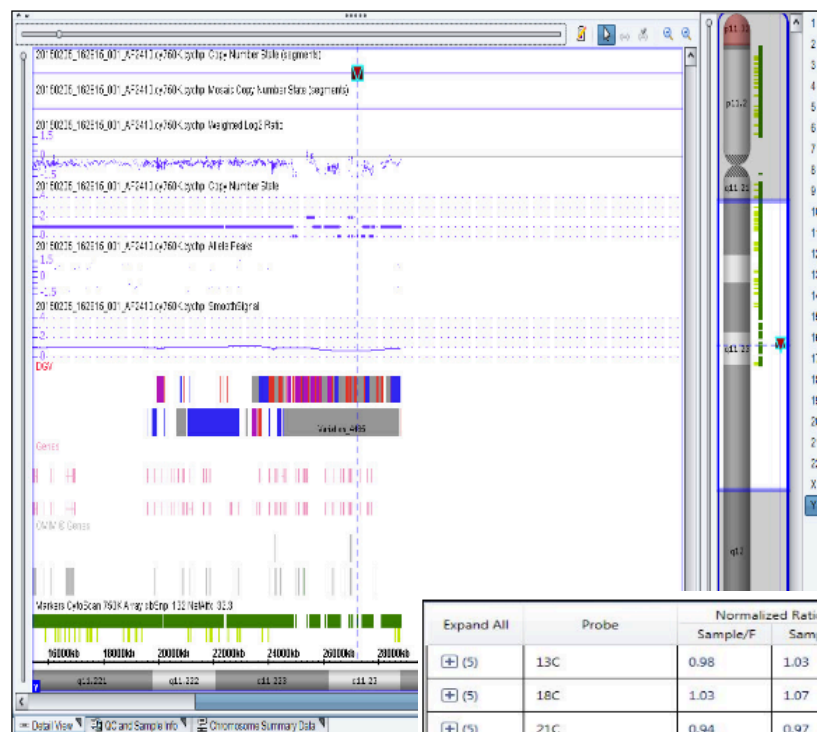
最后的答案




























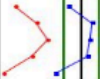


- 父亲存在一个1q21.1区域的缺失，遗传给孩子
- 母亲存在一个同区域RBM8A基因第6号外显子区域隐蔽的缺失，也遗传给孩子
- 造成胎儿双侧挠骨缺失的隐性遗传病发病（二次）
- 将来准备通过PGD进行下一次的妊娠



病例 意外发现

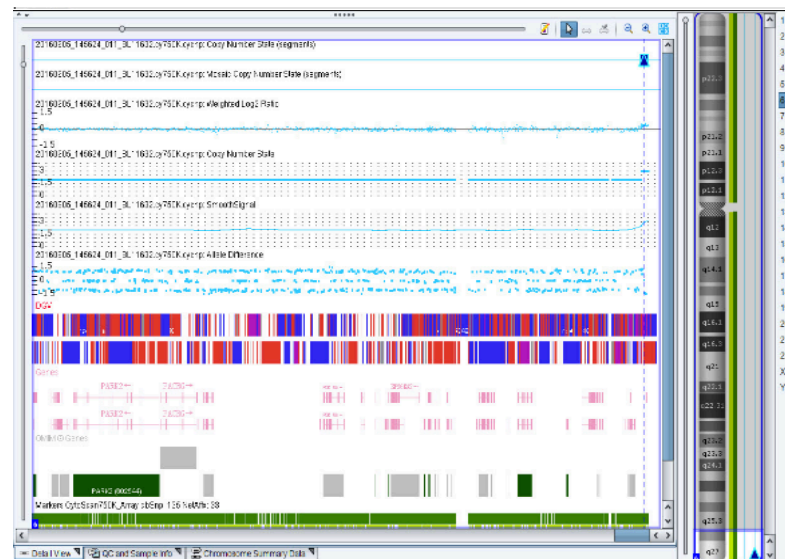
- 38岁高龄孕妇进行产前诊断
- 胎儿Y染色体Yq11.23区段存在415.8Kb片段的缺失，内含DAZ3,DAZ2基因，位于AZFc区域
- BoBs芯片诊断支持该结果
- 该胎儿存在男性不育的可能



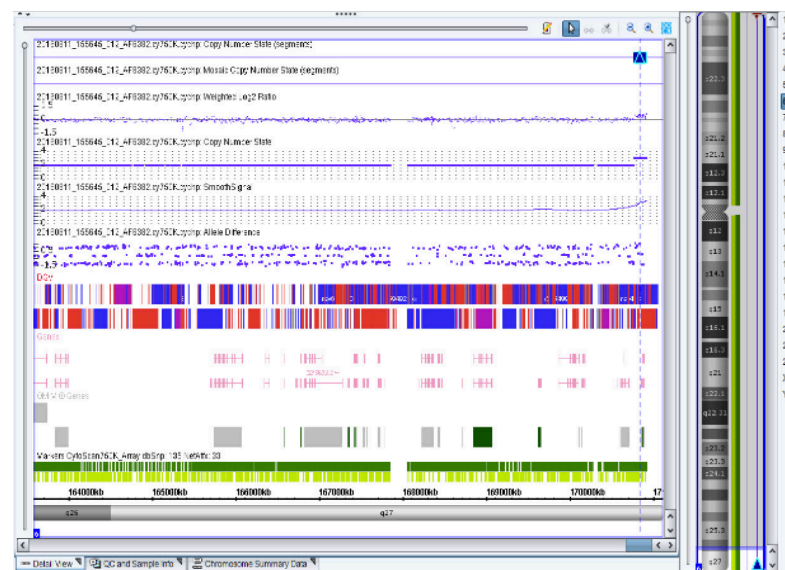
| Expand All | Probe | Normalized Ratios | | Threshold: 0.89-1.11 | | Threshold Y: 0.84-1.16 | | |
|---|--------------|-------------------|----------|----------------------|---|---|-----|-----|
| | | Sample/F | Sample/M | 0.0 | 0.5 | 1.0 | 1.5 | 2.0 |
|  (5) | 13C | 0.98 | 1.03 | | |  | | |
|  (5) | 18C | 1.03 | 1.07 | | |  | | |
|  (5) | 21C | 0.94 | 0.97 | | |  | | |
|  (6) | AUTO | 1.02 | 1.02 | | |  | | |
|  (8) | CDC | 0.97 | 0.99 | | |  | | |
|  (4) | DGS | 0.94 | 1.02 | | |  | | |
|  (4) | DiG | 0.99 | 1.01 | | |  | | |
|  (7) | LGS | 1.00 | 1.01 | | |  | | |
|  (6) | MDS | 0.97 | 0.99 | | |  | | |
|  (7) | PWS | 0.99 | 1.01 | | |  | | |
|  (4) | SMS | 0.96 | 0.98 | | |  | | |
|  (5) | WBS | 0.91 | 0.93 | | |  | | |
|  (5) | WHS | 0.97 | 0.99 | | |  | | |
|  | XC1: 79 Xp22 | 0.60 | 0.93 | |  | | | |
| | XC2: 80 Xp22 | 0.73 | 1.07 | | | | | |
| | XC3: 81 Xp21 | 0.80 | 1.06 | | | | | |
| | XC4: 84 Xq13 | 0.70 | 1.04 | | | | | |
| | XC5: 85 Xq27 | 0.53 | 0.85 | | | | | |
|  | YC1: 86 Yp11 | 3.44 | 1.05 | |  | | | |
| | YC2: 96 Yq11 | 3.71 | 1.05 | | | | | |
| | YC3: 87 Yq11 | 4.56 | 1.10 | | | | | |
| | YC4: 88 Yq11 | 2.74 | 0.59 | | | | | |
| | YC5: 89 Yq11 | 2.10 | 1.15 | | | | | |

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

分子诊断时代核型分析的价值

刘X，因NIPT提示“性染色体减少”行产前诊断

FISH检查结果：

未培养细胞 X0:XY:XYY=28:21:100

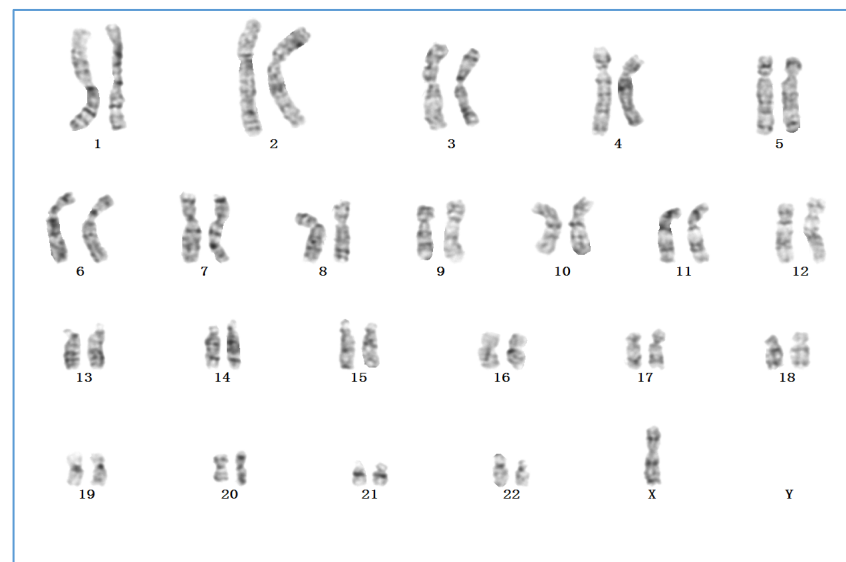
培养后细胞 X0[50]

细胞培养染色体检查结果: 45,X0[28]

SNP Array 检测结果： arr[hg19] Xp22.33(168,551-675,848)×0 or Yp11.32(118,551-625,848)×0

超声检查结果：

2017.1.3 检查示胎儿双顶径72mm,一侧股骨长47mm;
胎心规律，144次/分；胎儿四腔心切面，左心略小。
双顶径估计妊娠29W+1，股骨长估计妊娠26W+2



分子诊断时代核型分析的价值

- 目前考虑胎儿不同组织（胚层）性染色体存在多种嵌合形式
- 有Y染色体缺失，也有Y染色体增多的情形
- 且这两种异常的数量基本相等
- 所以在Microarray检测中，未发现Y染色体有含量上的变化
- 导致Turner综合征（45, X）的漏诊
- 但同时Microarray诊断的性染色体上拟长染色体区域的缺失也是病理性的（心脏的异常可能与其相关），该种异常也被核型分析所遗漏



引产后外生殖器模糊，心脏多发畸形

NIPT提示5号染色体减少病例的解析

32岁经产妇，有过一次胎儿小头畸形引产

本次NIPT提示“5号染色体减少”

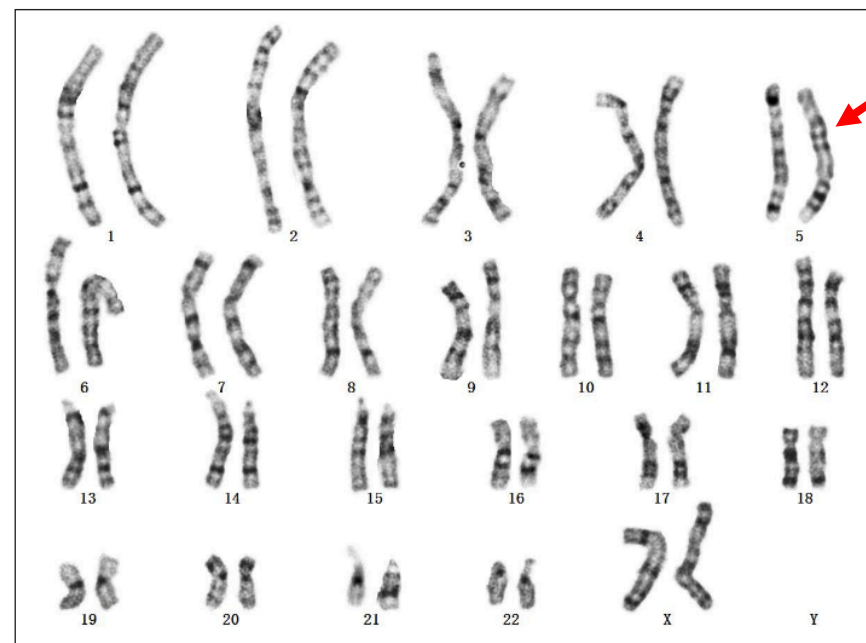
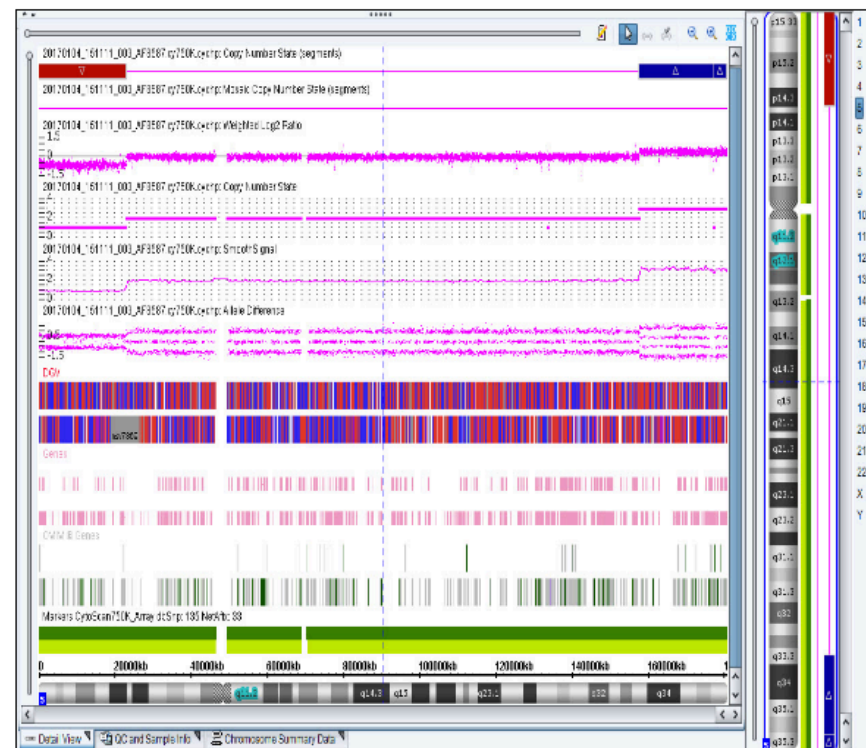
羊水穿刺全基因组芯片：

- 5p15.33p14.3区段存在22.8Mb片段的缺失（猫叫综合征）
- 5q33.3q35.3区段存在23.0Mb片段的重复

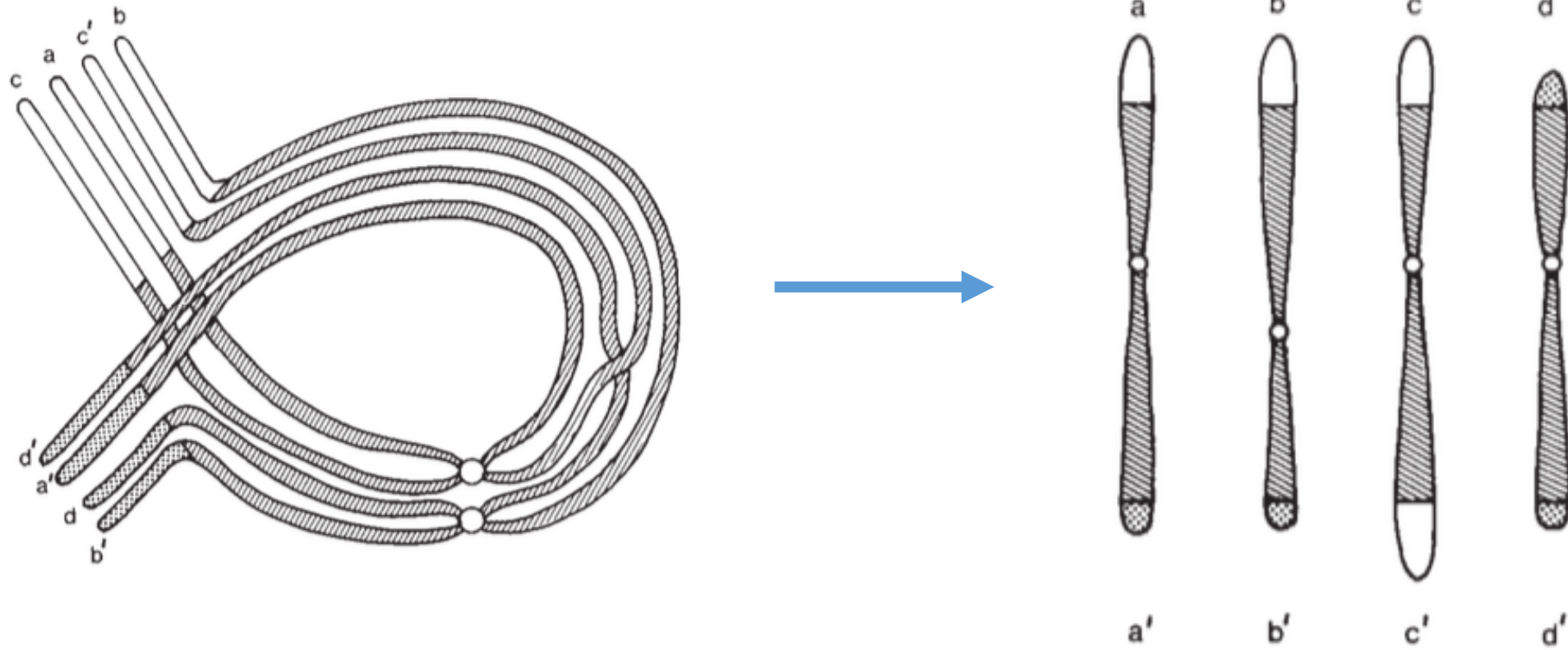
胎儿核型分析：46, XN, del (5)

父母核型分析：孕妇为46,XX,inv(5)(p14.3q33.3)
丈夫正常

孕妇：46,XX,inv(5)(p14.3q33.3)



5号臂间倒位引起后代的染色体内重复-缺失



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

欢迎交流 开放提问



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