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分子时代产前筛查和产前诊断技术和理念的变迁及发展

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摘要 最初的产前诊断主要针对染色体非整倍体, 随着遗传学检测技术的进展, 越来越多的遗传性疾病可以在产前得到诊断, 包括致病性拷贝数变异、单基因病、单亲二体等, 产前遗传学检测从细胞遗传学时代进入到分子时代. 随着cfDNA(cell-free DNA)检测迅速整合进入产前筛查领域, 产前筛查也从基于生化指标的母血清学筛查时代进入到分子时代, 很多单基因遗传病也可以通过扩展型携带者筛查被检出. 技术的进步极大地促进了学科的发展, 同时也极大地改变了人们的理念以及对产前筛查和产前诊断的认知.

关键词 产前筛查, 产前诊断, 染色体非整倍体, cfDNA检测, 染色体微阵列分析, 外显子测序, 孕妇高龄

最初的产前诊断主要针对染色体非整倍体, 随着遗传学检测技术的进展, 越来越多的遗传性疾病可以在产前得到诊断, 包括致病性拷贝数变异(copy number variant, CNV)、单基因病、单亲二体(uniparental disomy, UPD)等, 产前遗传学检测从细胞遗传学时代进入到分子时代. 随着游离DNA(cell-free DNA, cfDNA)检测迅速整合进入产前筛查领域, 产前筛查也从基于生化指标的母血清学筛查时代进入到分子时代, 很多单基因遗传病也可以通过扩展型携带者筛查被检出. 技术的进步极大地促进了学科的发展, 同时也极大地改变了人们的理念以及对于产前筛查和产前诊断的认知. 本文拟从检测技术发展的角度出发, 对产前筛查和产前诊断的认知和理念的变迁进行阐述和评价.

1 产前筛查的发展

产前筛查遵循的原则是在Wilson和Junger提出的筛查十大原则基础上完善而成的[1]. 对目标疾病而言, 所筛查疾病应具有重要的临床意义, 定义明确, 对公共健康具有重要影响; 对寿命或生活质量影响显著; 自然病史中存在无症状期, 使发现疾病成为可能; 通过在无症状期识别或治疗疾病, 结局能够得到改善. 对筛查性检测而言, 其本身应当安全、可被接受; 足够敏感和特异, 能够检出疾病并使假阳性率降至最低; 效价比高. 对被筛查的人群而言, 目标疾病的患病率应足够高; 所有人能够公平地得到检测和治疗; 检出的患者愿意接受进一步的诊断或治疗[2]. 基于上述原则, 产前筛查的目标疾病从最初的开放性神经管缺陷(neural

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tube defect, NTD), 到胎儿常见染色体非整倍体、单基因病, 至今已历经40余年(图1).

1.1 开放性神经管缺陷

1970年代, 英国开展了针对甲胎蛋白(α-fetoprotein, AFP)的多中心协作研究[3], 发现在孕16~18周, 怀有无脑儿的孕妇血清AFP水平显著升高, 由此, 针对开放性NTD的中孕期母血清AFP筛查成为第一个产前筛查检测项目.

通过超声检查可以直接观察到胎儿脊柱, 颅脑的"柠檬征"和"香蕉征"则提示胎儿可能罹患脊柱裂. 荟萃分析发现, 通过超声筛查脊柱裂的检出率为90%~95%, 假阳性病例罕见[4]. 另一方面, 围孕期补充叶酸使得NTD的发病率大大降低, AFP筛查的阳性预测值也随之下降. 越来越多的机构摒弃AFP筛查, 直接采用超声筛查NTD.

1.2 胎儿常见染色体非整倍体

胎儿常见染色体非整倍体指的是21、13和18三体. 根据筛查原理的不同, 可将其分为2类: (ⅰ) 基于母血清生化指标的产前筛查(maternal serum screening, MSS). (ⅱ) 基于胎儿cfDNA的产前筛查, 即无创产前检测(non-invasive prenatal testing, NIPT).

(1) MSS. 1984年, Merkatz等人[5]发现, 怀有21三体胎儿的孕妇血清AFP水平降低, 由此开启了针对胎儿21三体综合征的中孕期MSS, 新的指标, 如人绒毛膜促性腺激素(human chorionic gonadotrophin, hCG)、未结合雌三醇(unconjugated estriol, uE3)、抑制素A(inhibin A)等不断被发现并纳入筛查方案, 诞生了中孕期二联、三联、四联筛查方案. 1990年代, 英国胎儿医学基金会发现, 孕11~13^{+6}周胎儿颈部透明层(nuchal translucency, NT)增厚与胎儿非整倍体相关[6], 并将NT测量与早孕期母血清hCG、妊娠相关血浆蛋白A(pregnancy associated plasma protein A, PAPP-A)检测相结合进行早孕期筛查, 使检出率提升到86%, 复合假阳性率6.7%[7]. 2000年后, 国际上陆续开展SURUSS、FASTER等研究对早、中孕期MSS的不同策略进行了系统评价[8,9]. 结论显示, NT是最好的单个筛查指标, 在双胎中具有与单胎相似的检出率. PAPP-A是筛查效能最好的血清学指标. 在5%假阳性率下, 早中孕期整合筛查、分层筛查的检出率均可在90%以上.

我国的MSS始于1998年前后, 北京协和医院于2008年牵头的多中心前瞻性研究, 获得了中国孕妇人群21三体综合征筛查的数据库, 确定了各项筛查指标的中位数值[10]. 2010年, 原卫生部组织专家编写并颁布了《胎儿常见染色体异常与开放性神经管缺陷的产前筛查与诊断技术标准》, 标志着中孕期MSS成为国内开展最为广泛的服务模式. 2006年之后, 随着NT筛查的广泛开展, 国内一些医疗机构开始进行早孕期筛查或整合、分层筛查.

(2) cfDNA检测. 1997年香港中文大学Lo等人[11]首次报道在母血中含有胎儿源性的cfDNA片段, 采用二代测序(next-generation sequencing, NGS)技术, 结合生物信息学分析, 即可筛查胎儿21、18、13三体. 2011年, Chiu等人[12]通过前瞻性研究证实该技术可用于胎儿21三体的产前筛查, 由此该项技术正式进入临床,

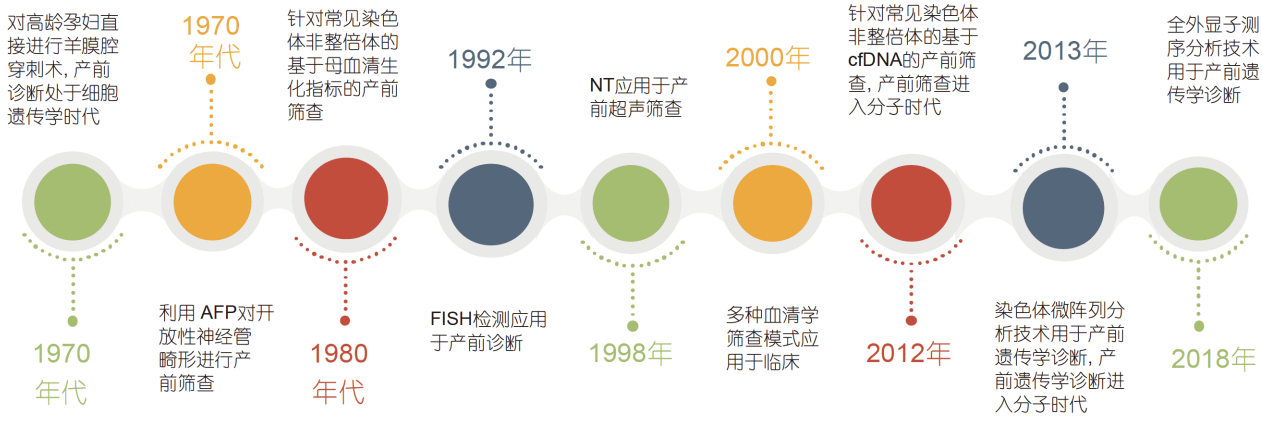

图 1 20世纪70年代至今的产前筛查与产前诊断技术变迁的时间轴线图
Figure 1 A timeline of changes in prenatal screening and prenatal diagnosis techniques from 1970s to the present

并迅速地被整合进入产前筛查系统. 由于这些“胎儿源性的cfDNA”来自胎盘细胞滋养细胞, 因此目前统一将这一筛查方法称为cfDNA检测.

早期的有关cfDNA检测的研究大多基于高风险人群[13~15], 针对普通孕妇人群的前瞻性研究不多. 2013年, 北京协和医院首次证实在低龄孕妇中, cfDNA检测可取得与高龄、高风险孕妇相似的检测效力[16]. 另一项具有代表性的多中心大样本前瞻性研究来自Norton等人[17], 证实在普通人群中cfDNA检测的筛查效率显著优于MSS. 在上述研究的基础上, 美国医学遗传学与基因组学学会(American College of Medical Genomics, ACMG)于2016年发布有关胎儿染色体非整倍体无创产前筛查的共识[18], 明确指出cfDNA检测是最敏感的对于胎儿21、18、13三体综合征产前筛查的技术手段. 最近的荟萃分析[19,20]显示, cfDNA检测对单胎妊娠21、18、13三体的检出率分别为99.7%、97.9%、99.0%, 是目前最有效的、应用最广泛的胎儿常见染色体非整倍体的产前筛查方法.

我国的cfDNA检测始于2011年, 全国产前诊断专家组以专家共识的形式在国际上率先提出cfDNA检测的临床定位应为胎儿常见染色体异常的产前筛查技术[21]. 此后, 通过2年的试点工作, 原国家卫生和计划生育委员会办公厅于2016年组织全国产前诊断专家组编写并发布了《孕妇外周血胎儿游离DNA产前筛查与诊断技术规范》, 为该技术在我国规范、有序地开展奠定了坚实的基础.

1.3　单基因病的携带者筛查

随着测序技术的发展, 单基因病的携带者筛查逐渐成为产前筛查的重要组成部分, 主要针对因认知和身体残疾影响生活质量的疾病, 使预期寿命缩短的疾病, 或通过药物、手术干预能够改善的疾病[22]. 德系犹太人自1971年起对Tay Sachs病进行携带者筛查, 使该病的发生率降低了90%以上[23]. 随着人口特征的改变, 混合种族家庭越来越多, 对普通人群进行数十甚至数百种基因病的扩展型携带者筛查(expanded carrier screening, ECS)的必要性越来越充分, NGS技术的进展也使ECS从技术上成为可能. 由于缺乏基于中国人群的设计良好的前瞻性研究, 以及被行业广泛接受的共识或指南, 目前国内尚未在临床上开展ECS.

2　产前遗传学诊断技术的发展

产前遗传学诊断是指利用绒毛、羊水、脐血等样本对胎儿遗传性疾病进行诊断. 随着遗传学技术的不断发展, 产前诊断技术领域也历经细胞遗传学诊断、细胞分子遗传学诊断和分子遗传学诊断三个阶段. 以染色体微阵列分析(chromosomal microarray analysis, CMA)和NGS技术为代表的分子遗传学诊断技术极大地推动了染色体病和单基因病的产前诊断(图1).

(1) 细胞遗传学诊断. 从1970年代到2010年, 产前遗传学检测基本上处于细胞遗传学阶段, 时至今日, G显带的染色体核型分析依然是产前细胞遗传学诊断的金标准, 但细胞培养耗时长, 通常只能检出10 Mb以上的片段改变.

(2) 细胞分子遗传学诊断. 细胞分子遗传学检测技术的出现引入了快速产前诊断的概念, 主要技术包括荧光原位杂交(fluorescence *in situ* hybridization, FISH)和荧光定量PCR(quantitative fluorescence PCR, QF-PCR), 用于快速检测胎儿13、18、21、性染色体有无数目异常, 通常可在1~3天获得结果, FISH与标准G显带核型分析的一致性在99%以上, 敏感性、特异性和预测值也都大于99%[24,25]. QF-PCR是对短串联重复序列(short tandem repeats, STRs)进行PCR扩增, 通过定性、定量分析STR的多态性来分析目标染色体有无数目异常[26~28].

上述两项技术的优点是无需进行细胞培养, 分析周期短, 尤其是QF-PCR检测, 通量高, 易于大规模开展. 我国从1990年代末开始开展这两项技术, 作为产前细胞遗传学诊断的补充检测, 有效地解决了产前诊断技术服务能力不足、诊断周期长等现实问题. 但上述技术也存在一定的局限性, 只能针对一定数目的片段进行分析, 存在一定的残余风险, 无法检出这些染色体的低水平嵌合、结构重排和标记染色体, 也不能提供有关其他染色体的信息[29,30]. 2015年, 我国产前诊断技术专家组对这两项技术在国内应用存在的具体问题进行了深入广泛的探讨, 并形成了其在产前诊断中应用的专家共识[31,32].

(3) 分子遗传学诊断. 无论是细胞遗传学检测还是分子细胞遗传学检测, 都存在局限性. 前者方法耗时、分辨率低, 而后者又不能做到全局分析. CMA检测能够在全基因组水平上进行扫描, 可检出小于100 kb大小的

CNV, 基本可以克服以上染色体分析技术的缺陷, 因此又被称为“分子核型分析”. 根据检测平台及其所检测出的CNV类型的不同, CMA技术可被分为两大类: 单核苷酸多态性微列阵(single nucleotide polymorphism array, SNP array)和基于微阵列芯片的比较基因组杂交(array-based comparative genomic hybridization, aCGH)技术, 目前大多采用CNV+SNP两者结合的芯片.

2010年5月, 国际细胞基因组芯片标准协作组(The International Standard Cytogenomic Array, ISCA Consortium)组织总结了21698例儿童遗传病的研究结果, 发现对于核型分析结果正常的病例, aCGH可检测出12.2%的致病性CNV, 从而推荐将aCGH作为对于未知原因的发育迟缓、智力低下、多种体征畸形以及自闭症的首选检测方法[33]. CMA技术在儿童遗传病诊断领域的成功应用, 使其很快就拓展到产前诊断领域[34~38]. 和传统的细胞遗传学检测相比, CMA检测最大的优势在于能够检出更小的不平衡性改变、无需细胞培养、自动化操作、快速得到结果. CMA在检出非整倍体方面具有100%的准确性, 对于有临床指征而核型正常的病例也可以提供更高的诊断率[34,39,40], 对于核型正常而超声发现胎儿结构异常的病例, CMA可以增加6%的诊断率[39]. 基于上述发现, 2013年美国妇产科医师协会(American College of Obstetrics and Gynecology, ACOG)的指南[41]和2014年国内的专家共识[42]均提出, 对于产前超声结构异常的病例, 应行包含CMA在内的产前遗传学诊断. 鉴于对于超声和染色体核型都正常的病例, CMA可以检出1.7%的病理性CNV[39], ACOG和美国母胎医学协会(Society for Maternal-Fetal Medicine, SMFM)在2016年发布实践指南, 进一步推荐对于所有选择进行侵入性产前诊断的患者都进行CMA检测[43]. 2018年国际产前诊断协会(International Society for Prenatal Diagnosis, ISPD)、SMFM、围产质量基金会(Perinatal Quality Foundation, PQF)发布的有关产前DNA测序的联合申明, 将CMA列为对于超声发现胎儿结构异常病例的产前标准遗传学诊断方法[44].

以外显子测序(exome sequencing, ES)技术为代表的杂交捕获和高通量测序技术近年来在胎儿超声结构异常的遗传学诊断领域得到应用. 根据检测范围的不同, ES技术可分为全外显子测序(whole exome sequencing, WES)和临床外显子测序(clinical exome sequencing, CES)两大类. ES最初主要用于儿童遗传病的诊断, Yang等人[45,46]对2000个家系进行诊断性ES检测, 在25.2%的病例中发现基因突变, 从而奠定了ES检测对于罕见孟德尔遗传单基因病的诊断价值. 和CMA检测一样, ES技术在儿童遗传病诊断领域的成功应用, 使其很快就拓展到产前诊断领域[47~50], 2019年发表在*Lancet*上的两篇分别来自欧洲和美国的两个大样本前瞻性的、针对背景人群的研究显示, 当核型分析和CMA检测均未有阳性发现的情况下, WES检测可检出10%~12.5%的单基因变异[51,52]. 在上述研究基础上, 2020年ACMG发布指南, 正式推荐对于超声发现胎儿结构异常且核型分析和CMA检测结果正常的病例, 应进一步行ES检测以排除胎儿单基因变异[53].

近年来国内一些具备单基因病产前诊断基础的医疗机构逐步开展WES检测, 取得了良好的效果, 预示着该项技术在我国很快会进入产前诊断临床应用领域[54,55].

3 产前诊断指征的变迁和发展

产前诊断通过羊膜腔穿刺术、绒毛取样(chorionic villus sampling, CVS)或脐静脉穿刺术来获取样本. 随着实时超声引导技术的引入, 侵入性产前诊断的操作相关性流产风险也随之降低, 2015年的一项荟萃分析显示, CVS和羊膜腔穿刺的操作相关性流产风险分别为1/450和1/900[56].

分子遗传学检测在临床的广泛应用使得人们的理念和对产前诊断指征的认知随之发生改变, 主要体现在以下三个方面: 孕妇高龄(advanced maternal age, AMA)、超声软指标、cfDNA检测发现的除21、13、18三体以外的其他染色体异常(以下简称cfDNA检测的意外发现).

3.1 AMA

在分子遗传学检测应用于临床之前, AMA是最常见的产前诊断指征. 胎儿21、13、18三体综合征的发生率随孕妇年龄的增加而增加[57~59]. 在1970年代, 标准的产前保健措施是对AMA孕妇提供羊膜腔穿刺术以检测胎儿是否存在染色体异常. 我国的《中华人民共和国母婴保健法》明确规定, 应对高龄初产妇进行产前诊断. 随着AMA孕妇的不断增加和MSS的广泛开展, 这一策略受到质疑[60~65].

cfDNA检测的广泛应用极大地改变了人们对产前筛查和诊断的认知. 2016年ACMG共识[18]以及我国的有关技术规范都明确指出, 应告知所有孕妇, cfDNA检测是最敏感的针对胎儿21、18、13三体综合征的产前筛查手段, AMA孕妇可以选择cfDNA检测, 而无需进行侵入性产前诊断. 孕妇年龄越大, cfDNA筛查的效率越高, 以21三体为例, 在妊娠10周, 当孕妇年龄为20、35、40岁时, cfDNA检测的阳性预测值(positive predictive value, PPV)分别为38%~80%、73%~95%、91%~99%. 对于胎儿18、13三体也是如此[66]. 这些研究结果更加说明对AMA孕妇采用cfDNA检测是有益的.

与孕妇年龄相关的胎儿染色体异常类型只是21、18和13三体综合征, 胎儿性染色体非整倍体和致病性CNV的发生率与孕妇年龄无关[39,67~69]. 系统性回顾和荟萃分析研究结果显示, 36岁以下孕妇的胎儿致病性CNV的发生率高于21三体的发生率[68]. 如果综合考虑胎儿染色体异常(如整条染色体非整倍体、致病性CNVs、UPD)的联合发生率, 当孕妇年龄为20岁时, 胎儿染色体异常的联合发生率为1/122, 当孕妇年龄为40岁时, 该发生率高达1/40[70].

基于上述研究结果, ACOG在2020年发布关于胎儿染色体异常的产前筛查指南[70], 指出任何孕妇都可以选择进行cfDNA检测或者是侵入性产前诊断, 而无论孕妇年龄多少岁, 如果选择后者, 则应进行包含CMA检测在内的遗传学检测. 至此, 在学术层面上, 对AMA孕妇直接进行侵入性产前诊断的策略已被摒弃, AMA不再是侵入性产前诊断的指征.

3.2　超声软指标

产前超声异常包括胎儿结构异常和超声软指标. 前者往往由于胎儿遗传性疾病所致, 其潜在机制包括染色体异常、致病性CNV、致病性单基因变异、UPD等, 此类病例已逐渐成为目前产前遗传学诊断最主要的指征[39,51,52,71~73].

超声软指标指的是一些特殊的超声特征, 其临床意义并不明确, 常常为一过性, 在晚孕期或出生后不久即自然消退, 大多数胎儿并无不良结局, 但这些软指标的存在与胎儿染色体异常和/或妊娠不良结局之间有一定关联, 具有一定的统计学意义. 在cfDNA检测应用于临床之前, 超声软指标一度被认为是重要的侵入性产前诊断指征. 事实上, 只有27%的21三体胎儿在中孕期超声检查中可以发现结构异常[74]. 2013年的一篇荟萃分析认为, 大多数孤立性中孕期软指标对胎儿21三体前设风险的校正很有限[75]. 如果孕妇cfDNA检测结果为阴性, 漏诊21三体的可能性非常低, 在这种情况下, 超声软指标对于21三体的预测价值非常有限.

CMA和ES检测技术在产前诊断领域的广泛应用再次刷新了人们对超声筛查的价值的认识. 以NT为例, 起初NT被认为是胎儿21三体综合征的标记物, 进一步研究发现, NT增厚和致病性CNV、某些遗传综合征以及胎死宫内等不良妊娠结局密切相关[76]. Choy等人[77]对50例NT>3.5 mm的病例进行基因组测序研究, 32%的病例获得了遗传学诊断结果, 其类型包括染色体异常、致病性CNV以及致病性单基因点突变. 他们指出, 对于NT增厚的病例, 应常规进行产前遗传学检测.

超声软指标还和妊娠不良结局具有相关性. Hurt等人[78,79]对Welsh地区2008~2011年所有产检孕妇的中孕期系统胎儿超声检查的结果进行前瞻性研究, 对其中具有超声软指标的病例进行追踪随访, 结果显示, 应结合既往胎儿染色体非整倍体筛查结果对超声软指标者进行个性化分析, 应随访胎儿生长情况、胎盘功能以及相关脏器的异常情况, 做出个性化的综合判断和处理.

综合以往的研究结果, ACOG在2020年关于胎儿染色体异常的筛查的指南对超声筛查的意义再次进行总结[70], 指出应对NT增厚和颈部水囊瘤病例进行详细的遗传咨询并提供产前遗传学检测. 该指南对中孕期超声软指标的处理也列出了个性化处理方案. 这一指南的发布, 对于超声软指标的临床处理策略具有很大的指导意义.

3.3　cfDNA检测的意外发现

cfDNA检测意外发现的发生率为0.12%~1.03%[80~83]. 原因大致可以分为以下三类: (i) 胎儿染色体异常; (ii) 局限性胎盘嵌合体(confined placental mosaicism, CPM); (iii) 母体CNV. 荷兰实验室的无创产前检测的评估研究(Trial by Dutch laboratories for Evaluation of Non-Invasive Prenatal Testing, TRIDENT)[81]对2527例cfDNA检测结果为高风险而进行诊断性检测的病例结果进行研究, cfDNA检测的意外发现占全部产前诊断的1.6%, 在这些意外发现的病例中,

24.4%为胎儿染色体异常, 53.6%为胎盘染色体异常(其中59.1%合并胎儿先天性异常或者严重的生长受限), 2.4%为母体染色体异常. 常家祯等人[84]对在北京协和医院进行cfDNA检测的25334例结果进行回顾性分析, 意外发现的发生率为0.79%, 其中70.8%的病例为性染色体非整倍体高风险, 29.2%的病例为罕见常染色体三体高风险. 上述研究结果显示, cfDNA检测的意外发现并不少见, 大部分病例都涉及到胎儿或胎盘的染色体异常, 应对此类病例进行侵入性产前诊断, 并重视妊娠结局随访.

4 总结与展望

综上所述, 随着遗传学检测技术的飞速发展, 无论是产前筛查还是产前诊断领域, 都从传统的细胞遗传学时代进入到分子时代, 检测的目标疾病也从单一的染色体非整倍体迅速扩展到致病性CNV、单基因病、UPD等领域, CMA检测已经成为标准遗传学检测. 技术的进步极大地改变了人们的理念以及对产前筛查和产前诊断的认知, 以往的观点被重新评价, 有些观点被摈弃. 强调每个孕妇都可以在知情同意的基础上选择是否进行侵入性诊断性检测, 在学术层面上, 孕妇高龄不再作为进行产前诊断的指标或依据. 在临床实践中, 要充分做好对孕妇的检测前和检测后咨询, 详细告知侵入性操作的并发症风险, 各项遗传学检测技术的性质、检测的目标疾病、局限性、检测周期、费用, 由孕妇在知情同意的基础上根据自身意愿自行选择.

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Development of the technologies and cognition of prenatal screening and prenatal diagnosis in an era of molecular genetics

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Original prenatal diagnostic testing focused primarily on chromosomal aneuploidies. Advances in genetics and genomic medicine have led to a dramatic increase in the availability of genetic testing, including in the prenatal period. Prenatal diagnosis is available for an ever-increasing number of disorders, including copy number variants, single gene disorders, and uniparental disomy. Prenatal genetic testing has stepped from the cytogenetic era into the molecular era. At the same time, prenatal screening has improved with development of cell-free DNA (cfDNA) screening, as well as expanded carrier screening for a broad array of inherited conditions. The advances in the technologies promote scientific research in this area and have altered people's cognition of prenatal screening and prenatal diagnosis.

prenatal screening, prenatal diagnosis, chromosomal aneuploidy, cfDNA testing, chromosomal microarray analysis, exome sequencing, advanced maternal age

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