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Review

New genetic testing in prenatal diagnosis

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SUMMARY

Determining a genetic diagnosis prenatally permits patients to make informed reproductive decisions and to be counseled about possible fetal outcomes. Therefore, it is important for the provider to be aware of the spectrum of genetic conditions and to use appropriate testing modality to obtain specific diagnosis. This article reviews genetic techniques available for prenatal diagnosis such as preimplantation genetic testing, chromosomal microarray, non-invasive prenatal screening, and next-generation sequencing. Chromosomal microarray has emerged as the first diagnostic test for evaluation of multiple congenital anomalies and developmental delay as most of the next-generation sequencing methods do not detect copy-number variants (CNVs). Exome sequencing and whole genome sequencing are time-consuming, so if this needs to be done to obtain an accurate genetic diagnosis, allow sufficient time.

1. Introduction

Prenatal diagnosis plays an essential role in contemporary obstetrical care, and with proper planning, it can be available for pregnancies at risk for chromosomal and single-gene disorders. Standard chromosome testing has been available since the 1960s [1] and has been commonly used in the prenatal setting, when a fetus has abnormal findings on ultrasound. Prenatal genetic testing has now become much more sophisticated with an improved level of resolution. Standard chromosome testing is being superseded by the use of chromosomal microarrays. Most forms of prenatal diagnosis require invasive procedures for fetal-sample collection and therefore, although considered safe, these procedures involve a risk of fetal loss. The presence of cell-free fetal DNA in maternal circulation has been used as a basis for the development of non-invasive prenatal testing [2]. Application of the latest technologies, such as next-generation sequencing, which features both high sensitivity and accuracy, is allowing for more accurate preconception counseling when there is a previously affected relative, thereby broadening the scope of prenatal diagnosis to include many diseases that are both paternally and maternally inherited.

Determining genetic diagnosis prenatally permits patients to make informed reproductive decisions and to be counseled about possible fetal outcomes, management options and recurrence risks. Therefore, it is important for the physician to be aware of the full spectrum of genetic conditions, and to use appropriate testing and referrals to genetic healthcare providers in order to obtain a specific diagnosis.

2. Preconception counseling

The prevalence of paternal and maternal conditions that are relevant to pregnancy outcomes varies according to many factors, such as parental age, ethnicity, medical history, and family history.

Family history plays a critical role in assessing the risk of inherited medical conditions and single-gene disorders [3]. In general practice, the family history can be obtained using a questionnaire or a three-generation pedigree. A family history screening allows stratification of the risk level. Also, the use of a family history screening has been shown to increase the likelihood of detecting a patient at high risk of developing an inherited medical condition by 20%, compared with medical record review alone [4]. Family history of developmental delay, congenital malformations, or other constellation of clinical findings suggestive of a genetic condition requires thorough evaluation. If a disorder in the individual's family has been identified as having a genetic cause, it may be possible to test parents to determine the risk for having an affected child. For example, a family history of known genetic conditions, such as Tay–Sachs or cystic fibrosis, should prompt testing for at least the person with an affected relative. It is paramount to identify the mutation in an affected individual, so prenatal diagnosis or preimplantation genetic diagnosis in cases of in-vitro fertilization (IVF) can be performed in order to test specifically for an identified familial mutation and decrease the risk of affected pregnancy. A history of recurrent pregnancy loss should

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prompt testing of both parents for chromosomal translocations. A parent with a balanced translocation identified by standard chromosome analysis can produce normal, balanced or unbalanced gametes that result in normal, balanced or unbalanced fetus. Recent data show that apparently balanced chromosomal rearrangements are associated with an abnormal phenotype in 6.7% of cases that may be due to cryptic genomic imbalances or to the disruption of genes at the breakpoints [5].

Whereas some genetic conditions that affect pregnancy outcomes are easily identified early in life, others are not and may require additional diagnostic testing. Up until about 10 years ago, ~65–70% of patients with congenital malformations remained undiagnosed, including many patients with multifactorial defects (e.g. non-syndromic cleft lip with or without cleft palate or neural tube defects) or polygenic defects (e.g. Hirschsprung disease or hypospadias), 15–25% were thought to be genetic or genomic, and 10% were thought to be environmentally determined [6]. Previously, the diagnostic yield for children with intellectual disability varied between 50% and 80%, with 17–47% having a genetic/genomic cause and 20–50% remaining undiagnosed [7]. With the advent of high-resolution chromosomal microarrays, an additional 10–15% of such patients receive a genomic diagnosis (which is often a sporadic occurrence in an otherwise normal family) [8,9]. As the use of exome sequencing has increased over the last 2 years, most laboratories are reporting an additional 25–40% success rate in obtaining a genetic diagnosis (some of which are also sporadic). The practical implication of all these recent developments is that with modern genomic/genetic diagnostic techniques, up to 70–80% of patients can receive a genetic/genomic diagnosis, with an additional 10% attributed to environmental influences and maternal conditions. Multifactoral defects such as isolated congenital heart defects or orofacial clefts have had a large number of genetic and environmental influences identified, but genetic diagnosis for multifactoral defects is not currently available for clinical use. Fortunately, recurrence risks are relatively low, in the 3–5% range, and this recurrence risk can be reduced by up to 50% through use of folic acid prior to conception and during early pregnancy (covered elsewhere in this volume). Exome sequencing can be quite useful for polygeneic or conditions manifesting genetic heterogeneity.

Ancestry influences the probability of being a carrier of many disorders that affect pregnancy. Typically, there is no family history of the condition as the carriers are asymptomatic. For example, using the current recommended mutation panel, negative carrier testing for cystic fibrosis in a Caucasian couple would result in a different chance of having an affected child than negative testing in an African-American couple since the carrier rate and the type of mutations are different in these two populations.

Parental age is an important risk factor for adverse pregnancy outcomes. The risk of chromosomal non-disjunction increases with increasing maternal age that translates into higher risk of trisomy 21, 13 and 18. Recent data emphasize the importance of paternal age as a risk factor for new dominant mutations. The male germline accumulates point mutations due to replication errors and reduced activity of repair enzymes, strands mispairing of short tandem repeats and longer exposure to environmental mutagens [10]. In addition, in human sperm DNA is more methylated than oocyte DNA, which may account for the greater number of paternally derived point mutations occurring within a CpG dinucleotide [11]. Because of the large number of cell divisions during spermatogenesis, the mutation rate for base substitutions is much higher in men than in women, and increases with paternal age. The risk for de-novo autosomal dominant mutations calculated by Friedman was 0.3–0.5% among the offspring of fathers aged >40 years [12]. Recently, with the use of whole-genome sequencing the increase in the rate of de-novo mutations has been estimated to be two mutations per year [13]. The conditions most strongly associated with advanced paternal age are those caused by point mutations in the *FGFR2*, *FGFR3* and *RET* genes and include Pfeiffer syndrome, Crouzon syndrome, Apert syndrome, achondroplasia as well as multiple endocrine neoplasia type 2A (MEN2A) and multiple endocrine neoplasia type 2B (MEN2B) [14]. Certain dominant conditions such as neurofibromatosis type 1 (NF-1) caused by point mutations or small deletions show a lesser association with paternal age. Some genetic conditions such as Noonan syndrome, Apert syndrome and MEN2B demonstrate germline mosaicism as a result of selection in male germline stem cells that explains an association of these conditions with advanced paternal age [15]. There is also a growing body of evidence that advanced paternal age is associated with an increased risk for complex disorders such as certain congenital anomalies, schizophrenia, and autism spectrum disorders [16,17]. For autosomes and sex chromosomes, there is no strong evidence that aneuploidy is significantly increased in newborns as paternal age increases but two possible exceptions are trisomy 21 and Klinefelter syndrome as recent data suggest a paternal effect, either acting alone or in combination with a maternal age effect [18].

Therefore, genetic risk stratification based on family history, ethnicity and parental age is paramount for preconception counseling. Increased awareness of the importance of using family history as a screening tool, and of the value of preventive measures and increased surveillance, can improve the outcomes. The availability of advanced technology allows for the identification of genetic etiologies in many conditions for which specific diagnostic testing is currently available.

3. Preimplantation genetic testing

Preimplantation genetic testing comprises all types of genetic testing performed on the embryos obtained from an IVF cycle and was first described by Handyside et al. [19] when the sex of the embryo was determined in two cases with a history X-linked genetic disorders. Preimplantation genetic testing is divided into two categories: preimplantation genetic screening (PGS) and preimplantation genetic diagnosis (PGD).

PGS is performed on the embryos obtained from the parents with presumed normal karyotypes. Chromosomal aneuploidy is a major factor in implantation failure and spontaneous abortions. It was demonstrated that half of the embryos produced in vitro had chromosomal abnormalities that significantly decreased the implantation rate [20]. PGS is used as an embryo screening method for aneuploidy, as the morphological analysis is not reliable for aneuploidy prediction. The most common method used for PGS is analysis of the embryonic cells on day 3 after fertilization with fluorescent in-situ hybridization (FISH). Fast turnaround times and high accuracy are the advantages of PGS with FISH but the limited number of chromosomes that can be evaluated is a significant limitation. Prospective trials of PGS by FISH have not demonstrated any improvement in pregnancy rates [21]. Comprehensive aneuploidy screening using whole-genome array showed that aneuploidy may occur in any of the 23 pairs of chromosomes. Recent retrospective studies showed that PGS using genome-wide approach and testing for all 23 pairs of chromosomes could improve the pregnancy outcome in certain groups or patients. Specifically, in women aged >35 years with a history of recurrent pregnancy loss, PGS is associated with reduced first-trimester spontaneous abortion rate [22]. However, prospective randomized trials have not demonstrated a definitive benefit of PGS and therefore, currently, PGS is not recommended for routine use.

PGD involves testing the embryos for a specific genetic disorder. PGD requires prior identification of the genetic cause of the