SOGC CLINICAL PRACTICE GUIDELINES

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Canadian Guidelines for Prenatal Diagnosis

GENETIC INDICATIONS FOR PRENATAL DIAGNOSIS

The following guidelines for prenatal diagnosis have been prepared by the Prenatal Diagnosis Committee of the Canadian College of Medical Geneticists (CCMG) and the Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada (SOGC) and approved by the Board of Directors of the CCMG and Executive and Council of the SOGC. These guidelines are an update of the guidelines previously published (Canadian College of Medical Geneticists and Society of Obstetricians and Gynaecologists of Canada, 1993). These guidelines will also be available on the Internet at ***www.sogc.org*** *and will be updated regularly.*

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FOR INFORMATION ON THE SELF-DIRECTED LEARNING EXERCISE SEE PAGE 540.

Abstract

Objective: To provide family practitioners, obstetricians, and geneticists with guidelines and recommendations for prenatal diagnosis.

Options: These guidelines apply to non-invasive screening techniques (including maternal serum screening and ultrasound) and to invasive techniques (including amniocentesis and chorionic villus sampling).

Outcomes: Improved prenatal diagnosis of congenital abnormalities, chromosomal anomalies or genetic conditions, and adverse outcomes related to prenatal testing procedures including pregnancy loss.

Evidence: The English language medical literature between 1976 and 2000 was reviewed, and opinions were obtained from experts in prenatal diagnosis. The level of evidence for the recommendations was determined using the criteria described by the Canadian Task Force on the Periodic Health Examination.

Benefits, harms, and costs: These guidelines will provide practitioners with a better understanding of the indications for prenatal diagnosis and the risks and limitations of available procedures.

Recommendations: Maternal age should be used to determine which women are at increased risk of having a child with a chromosomal anomaly. (II-2 A) Screening tests such as maternal serum screening could be used to modify a woman's age-related risks. (II-2 A) Amniocentesis should be offered to women at increased risk. (I A) Chorionic villus sampling can be offered as an alternative to amniocentesis. (I A)

Validation: These guidelines update the 1993 "Canadian Guidelines for Prenatal Diagnosis of Genetic Disorders." Recommendations were reviewed and revised by the Prenatal Diagnosis Committee of the Canadian College of Medical Geneticists and the Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada (SOGC), and were approved by the SOGC Council.

Sponsors: The Canadian College of Medical Geneticists and the Society of Obstetricians and Gynaecologists of Canada.

INCREASED RISK FOR CHROMOSOME ABNORMALITIES

In the Canadian health care system, invasive prenatal genetic testing is not generally offered to all women. Guidelines for access to testing are an attempt to balance genetic risks against procedural risks and economic considerations. Several screening techniques are currently employed to determine whether a couple is at increased risk to have a child with a chromosomal imbalance.[1]

The current standard for chromosome testing involves cell culture and evaluation of all chromosomes by a banding method (usually G banding) after an invasive procedure such as amniocentesis, chorionic villus sampling (CVS) or fetal blood sampling. In addition, based on the clinical indication for testing, syndrome-specific fluorescence *in situ* hybridization (FISH) probes for microdeletion detection (as in velocardiofacial/DiGeorge) may be used.

Interphase FISH testing for common trisomies and sex chromosome abnormalities may be appropriate to provide a rapid response when the patient is referred relatively late (after 21 weeks) or multiple congenital abnormalities are identified by ultrasound. In this circumstance, the patient and physician must be made aware of the limitations of interphase testing and that structural chromosome abnormalities and rare trisomies will not be detected.[2]

Prenatal tests can be divided into two categories: screening tests and diagnostic tests. Examples of screening tests for chromosome abnormalities include asking a woman's age, maternal serum screening, and ultrasound examination. Diagnostic testing includes CVS, amniocentesis, and cordocentesis.

MATERNAL AGE

The traditional recommendation is that all women who will be 35 years of age or older on the estimated date of delivery should be offered invasive prenatal testing,[3] but maternal age alone is a relatively poor predictor of fetal chromosomal abnormalities. Where facilities exist for additional screening methods, such as maternal serum screening, estimation of risk based on maternal age in isolation may not be appropriate. For example, a 35-year-old woman with a calculated risk equivalent to a 20-year-old may not be offered amniocentesis. Individual centres should determine their own policies on this issue (see next section).

Some centres may choose to offer amniocentesis for younger women carrying twins. The chance of a 32-year-old woman, who carries twins of unknown zygosity, having at least one child with Down syndrome is equivalent to the risks of a 35-year-old with a singleton pregnancy.[4] The chance of an abnormality must be balanced against the risks of the procedure in a twin pregnancy, which may be at least double that of an amniocentesis in a singleton pregnancy.[5]

BIOCHEMICAL MARKERS (MATERNAL SERUM SCREENING)

Certain biochemical markers in maternal serum measured in the first or second trimester can refine the age-related risk for trisomy 21 and trisomy 18. The most commonly used maternal markers include maternal serum α-fetoprotein (MSAFP), unconjugated estriol, and human chorionic gonadotropin (hCG) measured in the second trimester.[6] This combination of markers can detect approximately 60 percent of cases of fetal Down syndrome with a false positive rate of approximately four percent.[7] Other markers include inhibin A[8] and urinary β-hCG-core fragment,[9] also measured in the second trimester, and pregnancy associated plasma protein-A (PAPPA) and free β-hCG in the first trimester.[10] Various combinations of these markers and nuchal translucency measurement have been investigated.[10] Some of these combinations appear to have the potential to increase the detection rate for Down syndrome while decreasing the false positive rate.[10,11]

Computer-generated risks can be calculated which take into account several factors including maternal age, gestational age, weight, insulin-dependent diabetes, and the biochemical results. If the calculated risk exceeds the chosen cut-off value, amniocentesis can be offered. A commonly chosen cut-off value is equivalent to the age-related risk of a 35-year-old (1 in 385 at term or 1 in 270 in the mid-second trimester). Different programs may choose different cut-offs depending on local resources. Screening for chromosomal anomalies based on biochemical markers should only be considered within a comprehensive screening and prenatal diagnosis program including interpretation, education, and follow-up counselling.[7]

Various disorders in the fetus are associated with low maternal serum estriol levels (see "Biochemical and Molecular Disorders").

PREGNANCY HISTORY

Previous abortus, stillbirth or livebirth with a trisomy or other chromosomal abnormality

Because the birth of a stillborn or liveborn infant with an aneuploidy is associated with an increased risk of recurrence, invasive prenatal genetic testing should be offered in all subsequent pregnancies.[12,13] It is assumed that this increased risk applies to couples following a prenatal diagnosis of an autosomal trisomy. One exception would be Turner syndrome, where the recurrence risk is not significantly increased.[14] The birth of a stillborn or liveborn infant with a *de novo* structural chromosomal anomaly (with normal parental karyotypes) is usually not associated with an increased risk of recurrence[14] but prenatal testing is offered because there is an increased risk of parental mosaicism. The spontaneous abortion of a *de novo* chromosomally abnormal conceptus is generally not associated with an increased risk of chromosome abnormalities in subsequent pregnancies.[15] An exception would be the finding of a potentially viable chromosomal anomaly such as trisomy 21.[15] Karyotyping of both partners is generally recommended for couples who have three or more pregnancy losses (or two or more losses where local resources permit).

Potentially transmissible chromosomal rearrangement

Where the pregnant woman or her partner is mosaic for a chromosomal abnormality or carries a chromosomal rearrangement, prenatal diagnosis should be offered. The actual risk of bearing a liveborn infant with an unbalanced chromosomal complement varies with the particular rearrangement, the sex of the carrier, and the method of ascertainment.[16-18] A genetic consultation is always recommended.

Several cases of uniparental disomy (UPD) have been documented involving carriers (either the parent or the fetus) of balanced Robertsonian translocations or supernumerary markers.[19] Appropriate studies should be performed, as UPD has been shown to have a clinical effect for the chromosomes involved.[20]

Relatives other than offspring with Down syndrome

Having one relative with Down syndrome does not itself constitute an indication for invasive prenatal diagnosis, but may warrant further evaluation. Standard trisomy 21 accounts for about 97 percent of all cases of Down syndrome, in which case invasive testing is not usually indicated. If chromosomal analysis can not be obtained from the affected relative, karyotype analysis should be offered if the affected relative is a brother or sister of the pregnant woman or her partner. If the affected relative is more distantly related, the risk for an affected fetus is not significantly increased above the population risk.[21] If the pregnant woman or her partner is found to be a carrier for a chromosomal translocation, invasive prenatal testing should be offered.

If an individual has two or more relatives with the trisomy type of Down syndrome, a genetic referral is recommended for consideration of prenatal testing.

X-linked disorders

Carriers and affected individuals of some X-linked disorders can be identified by biochemical or molecular methods (see "Biochemical and Molecular Disorders"). Fetal sexing may be offered for disorders where no biochemical or molecular markers are available to confirm carrier status or identify an affected male. Molecular or chromosomal analysis of CVS or amniotic fluid is recommended.

Fragile X syndrome

Testing for fragile X syndrome is no longer recommended by cytogenetic methods, and molecular testing is now standard.[22]

Syndromes with elevated chromosomal breakage or other cytogenetic aberrations

Prenatal diagnosis for the following disorders requires special laboratory techniques; therefore, referral to a genetic centre prior to pregnancy is strongly recommended:

- fanconi anemia
- Bloom syndrome
- ataxia telangiectasia
- xeroderma pigmentosum
- Robert's syndrome

Therapeutic radiation

Exposure to therapeutic radiation in males is associated with a significant increase in both numerical and structural chromosomal abnormalities in sperm, even years after treatment.[23] However, there is no evidence that eggs exposed to

therapeutic radiation are similarly affected. Referral to a local genetics centre for evaluation is recommended.

Infertility treated with intracytoplasmic sperm injection

Sex chromosome abnormalities have been reported in about one percent of pregnancies following intracytoplasmic sperm injection (ICSI).[24] It would therefore be prudent to offer prenatal diagnosis for pregnancies conceived by ICSI.

Microdeletion/microduplication syndromes

Several microdeletion or microduplication syndromes have been identified, including DiGeorge/Shprintzen syndrome/ conotruncal heart defects (22q deletion), Beckwith-Wiedeman syndrome (11p duplication), and Prader-Willi/ Angelman syndrome (15q deletion). Although occasionally recognized by standard cytogenetic testing, FISH or molecular studies are generally required for diagnosis. Recurrence risks for affected patients or for the parents of an affected child depend on the specific syndrome and mechanism involved. Genetic counselling is recommended and prenatal testing should be offered for all at-risk individuals.

Abnormal Ultrasound Scan

i) Major fetal anomalies

A genetic assessment is recommended when major fetal abnormalities are detected by an ultrasound scan. Chromosomal abnormalities are frequently found in such cases, particularly with multiple congenital anomalies, neural tube defects, cystic hygroma, limb abnormalities, omphalocele, duodenal stenosis/atresia, significant ventriculomegaly or facial abnormalities,[25] or in association with intrauterine growth retardation or variation in amniotic fluid volume. FISH testing for a 22q11 deletion should be considered if there is prenatal detection of a fetal cardiac anomaly, especially of the conotruncal type.

ii) Sonographic markers of aneuploidy/ minor fetal anomalies

Several minor fetal anomalies or "soft signs" have been found to be statistically associated with fetal chromosomal anomalies. An increased risk for fetal Down syndrome is indicated by many second trimester soft signs including: increased nuchal thickening,[26] renal pyelectasis,[27] shortened femurs,[26] echogenic bowel,[26] echogenic foci of the left ventricle,[28,29] increased fetal iliac angle,[30] and hypoplasia of the middle phalanx of the fifth digit.[31] A slightly increased risk for fetal trisomy 18 but not fetal Down syndrome is associated with choroid plexus cysts.[32-34] A recent review suggested that a choriod plexus cyst as an isolated finding, with no other anomaly identified on ultrasound after an expert evaluation, increased the base risk of trisomy 18 by a factor of 7.09.[35] The positive predictive value of any of these markers is low and controversy exists around the relative value of each of these soft signs in detecting or excluding fetal chromosomal anomalies.

Determination of risk by a combination of maternal age and fetal nuchal-translucency thickness, measured by ultrasonography at 10 to 14 weeks under standard conditions, allows the detection of 72 percent of Down syndrome fetuses with a false positive rate of about five percent.[36]

Prediction of the risk for fetal trisomies based on soft signs should conform to accepted criteria for a screening program and should only be done where facilities exist for adequate follow-up. It is not clear how these ultrasound soft signs can be combined with other information such as maternal age or maternal serum screening to provide risk estimates. More studies are needed in this area.

NEURAL TUBE DEFECTS

The spectrum of neural tube defects (NTDs) includes anencephaly, spina bifida, encephalocele, and multiple vertebral defects. Occult spinal dysraphism associated with signs and symptoms, such as pigmented or hairy patch, bladder incontinence or hypoplastic foot or leg, should be considered as an NTD for risk calculations. Spina bifida occulta, usually found as an incidental radiologic finding of the absence of one or two vertebral arches, occurs in about five percent of the general population and should not be considered a risk factor for NTDs.[37] The risk of recurrence of NTDs varies according to the frequency in the general population as well as the family history, nutritional factors such as folic acid deficiency, and exposure to such drugs as valproic acid and carbamazepine, among other syndromic and non-syndromic factors. Assuming a population frequency for NTDs of one per 1000, the approximate risk of having a child with an NTD in the following situations is: affected sibling one to three percent, affected first cousin (maternal aunt's child) one percent, affected first cousin of another type 0.3 percent, mother on valproic acid one to two percent.[38]

Tools for prenatal diagnosis of NTDs include detailed ultrasound examinations and measurements of MSAFP, amniotic fluid α-fetoprotein (AFAFP), and amniotic fluid acetylcholinesterase (AChE). Women at increased risk of having a child with an NTD should be offered MSAFP testing and an ultrasound examination. Amniocentesis should be considered as a follow-up investigation for women at increased risk if local experience or technical factors preclude a reliable ultrasound evaluation for fetal spina bifida.

MATERNAL SERUM α-FETOPROTEIN

Elevated levels of MSAFP are associated with an increased risk of fetal NTDs. MSAFP screening at 15 to 18 weeks gestation can detect 71 to 92 percent of open NTDs with a

false positive rate of 1.2 to 3.9 percent.[39] MSAFP levels are reported as multiples of the median with each laboratory choosing its own cut-off value: commonly used cut-offs range between 2.0 and 2.5 multiples of the median MSAFP. Levels are adjusted for various factors including gestational age, maternal weight, maternal ethnic origin, and diabetic status. MSAFP screening should only be undertaken if facilities exist for adequate patient counselling and follow-up, such as ultrasound evaluation and additional counselling. Elevated MSAFP levels are also seen with other fetal anomalies including abdominal wall defects, skin disorders, and congenital nephrosis, as well as multiple gestation pregnancies, fetal demise, subchorionic hematoma, and underestimation of gestation. Unexplained elevations of MSAFP levels are associated with an increased risk of fetal growth retardation, oligohydramnios, later fetal demise, and maternal preeclampsia.[40,41] Amniocentesis should be considered as a follow-up investigation for an elevated MSAFP level if local experience or technical factors preclude a reliable ultrasound evaluation for fetal spina bifida. Some authors have suggested that an elevated MSAFP level is a risk factor for fetal cytogenetic abnormalities.[40] The data on this is limited and conflicting.[41] If amniocentesis is being done for investigation of an elevated MSAFP level, it may be prudent to do cytogenic studies.

AMNIOTIC FLUID α-FETOPROTEIN AND AMNIOTIC FLUID ACETYLCHOLINESTERASE

The detection rate for NTD by measurement of AFAFP is highest if amniotic fluid is sampled between 16 and 18 weeks gestation when 99 percent of open neural tubes can be detected. Testing can still be reliably done, however, between 15 and 21 weeks gestation. AFAFP can also be elevated with other fetal conditions including ventral wall defects, skin disorders, and congenital nephrosis. AFAFP measurements should be routinely made on all amniotic fluid samples obtained at the appropriate gestation, regardless of the indication for testing. AChE testing should be done if an elevated AFAFP level is found, as this factor is more specific for NTDs.

BIOCHEMICAL AND MOLECULAR DISORDERS

Prenatal testing is available for numerous metabolic and other single gene disorders. Couples at risk may be identified because of a previously affected offspring, a positive family history or by heterozygote screening. Determination of a couple's risk status should ideally be done prior to conception. The approach to prenatal diagnosis for biochemical and molecular diagnoses varies for each disorder. With few exceptions, a full genetic assessment is required to determine whether prenatal diagnosis is available to a particular family. If the diagnosis uses linkage rather than direct mutation analysis, extensive family investigation may be required before this question can be answered. Linkage analysis is dependent upon an accurate clinical diagnosis in affected relatives and correct family relationships, including possible instances of non-paternity. Before proceeding with a biochemical or molecular prenatal diagnosis, it is necessary to determine the appropriate diagnostic tests, the location of the appropriate testing laboratories, and the optimal tissues to be obtained. A medical genetics consultation is required for all families in this category.*

CARRIER SCREENING

Screening for the heterozygote or carrier state is recommended for individuals belonging to population groups known to have an increased risk for carrying certain genetic disorders. We strongly recommend that testing be done prior to pregnancy in order to allow genetic counselling and to arrange for prenatal testing if appropriate. If one partner is found to be a carrier, the other should be tested as soon as possible. If the woman is a carrier and the man cannot be tested, prenatal diagnosis may still be considered, since the risk to the fetus is at least one percent.

TAY-SACHS DISEASE

The carrier frequency for Tay-Sachs disease is one in 30 among Ashkenazi Jews and one in 14 among French Canadians in Eastern Quebec.[42,43] The frequency outside this region of Quebec is much lower (one in 41 to one in 98).[44] The carrier state can be detected by measuring serum hexosaminidase A (Hex A) activity. In pregnant women, carrier detection requires the measurement of enzyme activity in leukocytes. If one partner is found to be a carrier of the Tay-Sachs gene, the other should be offered screening as well, since mutations of the locus can also be found in other populations.

Three mutations account for 98 percent of disease-causing alleles in the Ashkenazi Jewish population. The molecular basis of disease in other populations is diverse. Screening is complicated by the presence of pseudodeficiency mutations that reduce the level of measured Hex A activity into the carrier range but do not confer a risk for disease. Two pseudodeficiency mutations have been identified.[45,46] When these mutations are taken into account, the carrier frequency in the non-Jewish population falls from one in

* Updated information on single gene disorders may be found at "Online Mendelian Inheritance in Man" at http://www.ncbi.nlm.nih.gov/omim .

167 to one in 277,[42] but remains the same in the Jewish population. DNA-based mutation analysis is recommended for all at-risk couples to assess their status for the pseudodeficiency alleles. Prenatal diagnosis is unnecessary if either parent is found to carry a known pseudodeficiency allele.

HEMOGLOBINOPATHIES

Adult hemoglobin (Hb A) is made up of 2 α- and 2 β-globin chains (α2β2). Each person normally has four normal α- and 2 β-globin genes. Thalassemia is caused by mutations in either the α-globin gene (α-thalassemia) or the β-globin gene (β-thalassemia), leading to decreased or absent α- or β-globin chains respectively. Persons who have inherited only one mutation (heterozygotes), known as thalassemia minor, are carriers but are asymptomatic.

Fetuses who are homozygous for the α^0-thalassemia deletions with a total lack of α-globin develop hydrops fetalis.[47] Women who are pregnant with these fetuses have an increased risk for serious maternal complications.[48] Infants who are homozygotes for β-thalassemia (β-thalassemia major) are normal at birth, but usually develop severe anemia within a year and require lifelong transfusions and nightly parenteral injections of an iron chelator.

Other globin gene mutations such as Hb E or Hb Lepore in combination with α-thalassemia trait can also cause severe anemia.[49] Sickle cell disease (Hb S) is caused by a specific mutation of the β-globin gene. Carriers for sickle cell disease are asymptomatic.[50] Homozygosity for Hb S is associated with increased risk for septicemia, strokes in childhood, and painful vaso-occlusive crises causing multiple organ damage in adults.[50] Other globin gene mutations in combination with Hb S can also cause severe sickling disorders.

Mutations for these hemoglobinopathies are common in people whose ancestors came from areas where malaria is endemic, including Africa, the Mediterranean basin, the Middle East, the Indian subcontinent, southeast Asia, and southern China. The carrier frequency in some populations may be 15 percent or more. In practice it has been recommended that everyone whose ancestors do not come from "northern Europe origin" should be considered at high risk.

A normal hemoglobin level alone does not rule out the carrier state for a hemoglobinopathy. The screening test for both α- and β-thalassemia is a low erythrocyte mean corpuscular volume of less than 80 fL. If this is found, additional tests should be carried out for a definitive diagnosis: serum ferritin to rule out iron deficiency, hemoglobin electrophoresis, and a Hb A2 and Hb F levels which are usually elevated in β-thalassemia. Hemoglobin H inclusion, if found, is indicative of α-thalassemia mutations. DNA analysis is often required for the diagnosis of α-thalassemia carriers. Furthermore, some persons are carriers for both α- and β-thalassemia mutations. For difficult cases, consultation with hematologists or geneticists is recommended. The definitive test for carriers of Hb S, C or D is hemoglobin electrophoresis, which should be offered to all couples of African or Caribbean descent. If the mean corpuscular volume is less than 80 fL, determination of Hb A2 is necessary to detect β-thalassemia trait. If a couple declines carrier testing or if the status of the infant is uncertain, the infant should be tested as soon as possible for sickling disorders. Early diagnosis and the proper use of prophylactic penicillin has been shown to be effective in reducing both morbidity and mortality.

CYSTIC FIBROSIS

Carrier testing is available by DNA testing for relatives of cystic fibrosis patients and their partners. Carrier testing should also be offered to both parents of a fetus with an ultrasound diagnosis of echogenic bowel taking into consideration their ethnic background.[51] In this context, echogenic bowel refers to bowel with an echogenicity similar to or greater than bone.[51] Carrier testing is not recommended for the general population at this time. Although the National Institutes of Health in the United States recently recommended cystic fibrosis carrier testing for the prenatal population,[†] the Canadian College of Medical Geneticists does not recommend this to be adopted or interpreted as a standard of practice at this time.

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