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Fragile X Testing in Obstetrics and Gynaecology in Canada

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

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Abstract

Objective: To provide Canadian family physicians, genetic counsellors, medical geneticists, midwives, and obstetrician-gynaecologists with recommendations regarding screening for fragile X in the obstetrical and gynaecological population.

Methods: Medline, the Cochrane Library, journals, and textbooks were searched for English-language articles, published between 1966 and March 2008, relating to fragile X testing outcomes. Search terms included fragile X, screening, prenatal testing, pregnancy outcome, premutation, trinucleotide repeats, and ovarian failure. All study types were reviewed. Randomized controlled trial results were considered evidence of the highest quality, followed by results of cohort studies. Key individual studies on which the recommendations are based are referenced. Supporting data for each recommendation are summarized with evaluative comments and references.

This document represents an abstraction of the information.

Evidence: The quality of evidence reported in this document has been described using the criteria outlined in the report of the Canadian Task Force on Preventive Health Care.

Recommendations

1. Any testing for fragile X syndrome must occur only following thorough counselling and with the informed consent of the woman to be tested. (III-A)
2. Fragile X testing is indicated for a woman with a family history of fragile X syndrome, fragile X tremor/ataxia syndrome, or premature ovarian failure (in more than one family member) if the pedigree structure indicates that she is at risk of inheriting the mutated gene. Referral to a medical geneticist for counselling and assessment should be considered in these cases. (II-2A)
3. Fragile X testing is indicated for women who have a personal history of autism or mental retardation/developmental delay of an unknown etiology or who have at least one male relative with these conditions within a three-generation pedigree. (II-2A)
4. Fragile X testing is indicated for women who have reproductive or fertility problems associated with an elevated level of follicle stimulating hormone before the age of 40. (III-A)
5. Prenatal fetal testing via chorionic villus sampling or amniocentesis should be offered to women who are confirmed to be carriers of a premutation or full mutation of the fragile X gene (FMR-1). (II-2A) Pre-implantation genetic diagnosis is available as another reproductive option. (III-A)
6. Population screening for fragile X syndrome for all women in the reproductive age-range is feasible. However, it should be considered only when there is a provincial/regional program that

Key to evidence statements and grading of recommendations, using the ranking of the Canadian Task Force on Preventive Health Care

Quality of Evidence Assessment*	Classification of Recommendations†
I: Evidence obtained from at least one properly randomized controlled trial	A. There is good evidence to recommend the clinical preventive action
II-1: Evidence from well-designed controlled trials without randomization	B. There is fair evidence to recommend the clinical preventive action
II-2: Evidence from well-designed cohort (prospective or retrospective) or case-control studies, preferably from more than one centre or research group	C. The existing evidence is conflicting and does not allow to make a recommendation for or against use of the clinical preventive action; however, other factors may influence decision-making
II-3: Evidence obtained from comparisons between times or places with or without the intervention. Dramatic results in uncontrolled experiments (such as the results of treatment with penicillin in the 1940s) could also be included in this category	D. There is fair evidence to recommend against the clinical preventive action
III: Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees	E. There is good evidence to recommend against the clinical preventive action
	L. There is insufficient evidence (in quantity or quality) to make a recommendation; however, other factors may influence decision-making

*The quality of evidence reported in these guidelines has been adapted from The Evaluation of Evidence criteria described in the Canadian Task Force on Preventive Health Care.[75]
†Recommendations included in these guidelines have been adapted from the Classification of Recommendations criteria described in the The Canadian Task Force on Preventive Health Care.[75]

can test and adequately counsel the targeted population about the meaning and implications of the results. (II-2B)

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INTRODUCTION

In 2002, the Genetics Committee and the Maternal Fetal Medicine Committee of the Society of Obstetricians and Gynaecologists of Canada noted that:

> A large amount of obstetric and genetic screening is undertaken once a woman is identified as being pregnant. Four areas that must be considered with regard to any form of screening in the pregnant (as well as the non-pregnant) population must include (1) opportunity for the counselling of the patient prior to the screening to assure informed choice, (2) the timing of the screening, (3) availability of laboratory technology, and (4) opportunity for diagnosis to occur in a timely fashion to allow the option of termination of pregnancy.[1]

Fragile X syndrome is an X-linked inherited condition caused by a mutation in the FMR-1 gene which maps to the long arm (q arm) of the X chromosome. It is one of the most common causes of inherited mental retardation[2–4] and affects approximately 1 in 4000 males and 1 in 8000 females.[3,5–9] Symptoms can be wide ranging, but invariably all males, and some carrier females, present with significant mental delay and behavioural problems.[3,6,7,8,10,11] The prevalence of women who are carriers of fragile X syndrome is estimated to be about 1 in 154 in a population without a family history of mental retardation, developmental problems, or autism, and 1 in 128 when a positive family history exists.[2]

The mutation leading to over 99% of cases of fragile X syndrome is an expansion in the number of copies of a sequence of nucleotides, consisting of CGG, referred to as an unstable repeat sequence in the gene known as FMR-1 gene.[3,4] There are four forms of the gene, each with a different number of copies of the repeated segment. They are referred to as normal or common (6–60 CGG repeats), "gray zone" or intermediate (41–60 CGG repeats), premutation (60–200 CGG repeats), and full mutation (> 200 CGG repeats).[3,28] Although the definitions of intermediate and premutation alleles are blurred, premutation is clinically reported when the CGG repeats are > 55.[3,12] The full mutation form of the gene is silenced, and no mRNA is produced. The lack of the gene product FMRP, an RNA-binding protein, is responsible for the mental retardation.[3,7–14]

Since males typically have only one X chromosome, a male with an FM will always have fragile X syndrome, but the manifestations vary significantly, even within members of a

ABBREVIATIONS

FM	full mutation
FXTAS	fragile X-associated tremor/ataxia syndrome
PM	premutation

single family. In females who carry an FM, the clinical manifestations are even more variable than in males, because they have two X chromosomes, one of which is inactive. The proportion of the cells with the active X chromosome carrying the fragile X mutation determines the clinical manifestations in females. However, the clinical manifestations cannot be predicted by routine testing.

Until recently, individuals with premutations were thought to be asymptomatic. However, some PM carriers, primarily males but also females, have been found to be at risk of developing late onset tremor/ataxia syndrome or psychological symptoms.[15–21] In addition, females who are PM carriers may develop premature ovarian failure (risk of approximately 21%), which shortens their reproductive capability.[22–26]

When a PM allele is transmitted from a carrier mother to her offspring, there is usually an increase in the CGG repeat size of the mutant allele. Thus, premutation women are at risk of having children with full mutations. Studies have demonstrated that the risk of PM female carriers having children affected with fragile X syndrome is related to the size of the PM carried by the woman.[2,7] Berkenstadt et al.[2] showed that the rate of allele expansion from maternal PM to fetal FM was 10% in patients without a family history of mental retardation, developmental problems, or autism, compared with 50% for those with a positive family history. The higher rate of expansion to FM in the latter group was accounted for by a higher number of repeats found in the PM carriers of this group, because the expansion rate did not differ between the groups for a given size. However, the expansion rate is even higher in women who have a proven family history of fragile X syndrome. This has to be taken into consideration when counselling women identified as being PM carriers about their risk of having a child with an FM.

Intermediate fragile X alleles have a small risk of expansion, estimated in one study to be 6.6%.[12] However, no intermediate allele has been reported as expanding to an FM. Carriers of intermediate alleles are not at risk of having an affected child and should not be offered invasive prenatal diagnosis for fragile X syndrome.

LABORATORY TESTING FOR FRAGILE X

DNA analysis is the method used for the diagnosis of fragile X syndrome and the identification of carriers. Testing consists of evaluating the size of the CGG trinucleotide repeat in the FMR-1 gene and detects over 99% of the cases. Testing is highly sensitive and highly specific when done in qualified facilities.[27] Results reliably classify individuals as being in the normal, intermediate, premutation, or full mutation category. Well-established laboratory technical standards have been published and can be reviewed for additional details.[27,28]

TESTING IN OBSTETRICS AND GYNAECOLOGY

Testing for fragile X syndrome should be offered to women who

1. Have mental retardation, autism, or ataxia
2. Have a family history of an individual with a confirmed PM or FM in the fragile X gene
3. Have a family history (within a three-generation pedigree) of autism or mental retardation/developmental delay of unknown etiology
4. Have a family history suggestive of FXTAS
5. Are experiencing reproductive or fertility problems associated with an elevated level of follicle stimulating hormone levels before the age of 40 or who have a family history that includes more than one female with premature ovarian failure.

POPULATION SCREENING FOR FRAGILE X OF PREGNANT WOMEN AND NON-PREGNANT WOMEN IN THE REPRODUCTIVE AGE RANGE

Several studies have examined the options of newborn screening[29–31] and population screening of all pregnant women for their fragile X status.[2,32–34] The severity of the condition, the high incidence in the general population, the impact of the condition on the family and society as a whole, and the high detection rate of fragile X (99%) makes screening for this condition in women in the reproductive age range desirable and feasible.[2,29–31,34] It also has been shown to be cost-effective.[33] However, this should be done only if resources are in place to test and adequately counsel the targeted population about the meaning and implications of the results. Women who are found to be carriers of a premutation allele are confronted with a significantly increased risk of premature ovarian failure.[25] In addition, these women are at increased risk of developing late onset FXTAS,[19] although the penetrance of this condition in carrier women is not known at this point. Finally, the identification of a carrier female implies that her family members are at increased risk of also being carriers, placing them at risk of developing FXTAS and at risk of having children and/or grandchildren with fragile X syndrome.[32]

PRENATAL TESTING

When a pregnant woman has been confirmed to have a premutation or full mutation in the FMR-1 gene, prenatal testing by either chorionic villus sampling or amniocentesis is available and should be offered as an option to the patient. Since the methylation status of the FMR-1 gene is

often not yet established in chorionic villi at the time of sampling, the chorionic villus sampling results must be interpreted with caution. Thus, counselling with respect to invasive testing should be provided by experienced personnel.[20,34]

Pre-implantation genetic diagnosis is available for at-risk couples who present before conception and should be discussed with them.

The quality of evidence reported in this document has been assessed using the Evaluation of Evidence criteria in the Report of the Canadian Task Force on Preventive Health Care (Table).[35]

Recommendations

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6. Population screening for fragile X syndrome for all women in the reproductive age-range is feasible. However, it should be considered only when there is a provincial/regional program that can test and adequately counsel the targeted population about the meaning and implications of the results. (II-2B)

REFERENCES

1. Wilson RD, Davies G, Désilets V, Reid GJ, Shaw D, Summers A, et al. Cystic fibrosis carrier testing in pregnancy in Canada. J Obstet Gynaecol Can 2002; 24:644–7.
2. Berkenstadt M, Ries-Levavi L, Cuckle H, Peleg L, Barkai G. Preconceptional and prenatal screening for fragile X syndrome: experience with 40,000 tests. Prenat Diagn 2007;27: 991–4.
3. Warren ST, Sherman SL. The fragile X syndrome. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. Metabolic basis of inherited disease. 8th ed. New York (NY): McGraw-Hill; 2001:1257–90.
4. Hagerman RI, Hagerman PJ, eds. Fragile X syndrome: diagnosis, treatment, and research, 3rd ed. Baltimore (MD): Johns Hopkins University Press; 2002.
5. Brown WT. Perspectives and molecular diagnosis of fragile X syndrome. Clin Lab Med 1995;15:859–75.
6. de Vries BB, van den Ouweland AM, Mohkamsing S, Duivenvoorden HJ, Mol E, Gelsema K, et al. Screening and diagnosis for the fragile X syndrome among the mentally retarded: an epidemiological and psychological survey. Collaborative Fragile X Study Group. Am J Hum Genet 1997;61:660–7.
7. Toledanoel-Vanagaite L, Magal N, Davidov B, Ehrlich S, Drasinover V, Taub E, et al. Fragile-X carrier screening and the prevalence of premutation and full-mutation carriers in Israel. Am J Hum Genet 2001;69:351–60.
8. Rousseau F, Rouillard P, Morel ML, Khandjian EW, Morgan K. Prevalence of carriers of premutation-size alleles of the FMR-1 gene—and implications for the population genetics of the fragile X syndrome. Am J Hum Genet 1995;57:1006–18.
9. Crawford DC, Acuna JM, Sherman SL. FMR-1 and the fragile X syndrome: human epidemiology review. Genet Med 2001;3:359–71.
10. Visootsak J, Warren ST, Anido A, Graham JM. Fragile X syndrome: an update and review for the primary pediatrician. Clin Pediatr 2005;44:371–81.
11. Fu YH, Kuhl DP, Pizzutti A, Pieretti M, Sutcliffe JF, Richards S, et al. Variation of CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. Cell 1991;67:1047–58.
12. Cronister A, Teicher J, Rohlfs EM, Donnenfeld A, Hallam S. Prevalence and instability of fragile X alleles: implications for offering fragile X prenatal diagnosis. Obstet Gynecol 2008;111:596–601.
13. Tarleton JC, Saul RA. Molecular genetic advances in fragile X syndrome. J Pediatr 1993;122:169–85.
14. Pearson CE, Nichol Edamura K, Cleary JD. Repeat instability: mechanisms of dynamic mutations. Nat Rev Genet 2005;6:729–42.
15. Hagerman PJ, Hagerman RJ. Fragile X-associated tremor/ataxia syndrome (FXTAS). Ment Retard Dev Disabil Res Rev 2004;10:25–30.
16. Willemsen R, Mientjes E, Oostra BA. FXTAS: a progressive neurologic syndrome associated with Fragile X permutation. Curr Neurol Neurosci Rep 2005;5:405–10.
17. Baba Y, Uitti RJ. Fragile X-associated tremor/ataxia syndrome and movements disorders. Curr Opin Neurol 2005;18:393–8.
18. Hessl D, Tassone R, Loesch DZ, Berry-Kravis E, Leehey MA, Gane LW, et al. Abnormal elevation of FMR-1 mRNA is associated with psychosocial symptoms in individuals with fragile X premutations. Am J Med Genet B Neuropsychiatr Genet 2005;139:115–21.
19. Coffey SM, Cook K, Tartaglia N, Tassone F, Nguyen DV, Pan R, et al. Expanded clinical phenotype of women with the FMR1 premutation. Am J Med Genet A 2008; 146:1009–16.
20. McConkie-Rosell A, Abrams L, Finucane B, Cronister A, Gane LW, Coffey SM, et al. Recommendations from multi-disciplinary focus groups on cascade testing and genetic counseling for fragile X-associated disorders. J Genet Couns 2007;16:593–606.
21. Loesch DZ, Churchyard A, Brotchie A. Evidence for, and a spectrum of, neurological involvement of carriers of the fragile X pre-mutation: FXTAS and beyond. Clin Genet 2005;67:412–7.
22. Kenneson A, Warren ST. The female and fragile X reviewed. Semin Reprod Med 2001;19:159–65.

23. Lin YS, Yang ML Familial premature ovarian failure in female premutated carriers of fragile X syndrome: a case report and literature review. Taiwan J Obstet Gynecol 2006;45:60–3.

24. Sullivan AK, Marcus M, Epstein MP, Allan EG, Anido AE, Paquin JJ, et al. association of FMR1 repeat size with ovarian dysfunction. Hum Reprod 2005;20:402–12.

25. Wittenberger MD, Hagerman RJ, Sherman SL, McConkie-Rosell A, Welt CK, Rebar RW, et al. The FMR1 premutation and reproduction. Fertil Steril 2007;87:456–65.

26. Sherman SL Premature ovarian failure in the fragile X syndrome. Am J Med Genet 2000;97:189–94.

27. Maddalena A, Richards CS, McGinniss MJ, Brothman A, Desnick RJ, Grier RE, et al. Technical standards and guidelines for fragile X: the first of a series of disease-specific supplements to the Standards and Guidelines for Clinical Genetics Laboratories of the American College of Medical Genetics. Quality Assurance Subcommittee of the Laboratory Practice Committee. Genet Med 2001;3:200–5.

28. Sherman S, Pletcher BA, Driscoll DA. Fragile X syndrome: diagnostic and carrier testing. Genet Med 2005;7:584–7.

29. Murray J Cuckle H, Taylor G, Hewison J. Screening for fragile X syndrome: information needs for health planners. J Med Screen 1997;4:60–94.

30. Murray J, Cuckle H, Taylor G, Hewison J. Screening for fragile X syndrome. Health Technol Assess 1997;1(4):i-iv,1–71.

31. Pembrey ME, Barnicoat AJ, Carmichael B, Bobrow M, Turner G. An assessment of screening strategies for fragile X syndrome in the UK. Health Technol Assess 2001;5:1–95.

32. Bailey DB Jr, Skinner D, Davis AM, Whitmarsh I, Powell C. Ethical, legal, and social concerns about expanded newborn screening: fragile X syndrome as a prototype for emerging issues. Pediatrics 2008;121:e693–704.

33. Musci TJ, Caughey AB. Cost-effectiveness analysis of prenatal population-based fragile X carrier screening. Am J Obstet Gynecol 2005;192:1905–12; discussion 1912–5.

34. ACOG Committee Opinion No. 338: Screening for fragile X syndrome. Obstet Gynecol 2006;107:1483–4.

35. Woolf SH, Battista RN, Angerson GM, Logan AG, Eel W. Canadian Task Force on Preventive Health Care. New grades for recommendations from the Canadian Task Force on Preventive Health Care. CMAJ 2003;169(3):207–8.