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POSITION STATEMENT

Practice guideline: joint CCMG-SOGC recommendations for the use of chromosomal microarray analysis for prenatal diagnosis and assessment of fetal loss in Canada

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ABSTRACT

Background The aim of this guideline is to provide updated recommendations for Canadian genetic counsellors, medical geneticists, maternal fetal medicine specialists, clinical laboratory geneticists and other practitioners regarding the use of chromosomal microarray analysis (CMA) for prenatal diagnosis. This guideline replaces the 2011 Society of Obstetricians and Gynaecologists of Canada (SOGC)-Canadian College of Medical Geneticists (CCMG) Joint Technical Update.

Methods A multidisciplinary group consisting of medical geneticists, genetic counsellors, maternal fetal medicine specialists and clinical laboratory geneticists was assembled to review existing literature and guidelines for use of CMA in prenatal care and to make recommendations relevant to the Canadian context. The statement was circulated for comment to the CCMG membership-at-large for feedback and, following incorporation of feedback, was approved by the CCMG Board of Directors on 5 June 2017 and the SOGC Board of Directors on 19 June 2017.

Results and conclusions Recommendations include but are not limited to: (1) CMA should be offered following a normal rapid aneuploidy screen when multiple fetal malformations are detected (II-1A) or for nuchal translucency (NT) ≥ 3.5 mm (II-2B) (recommendation 1); (2) a professional with expertise in prenatal chromosomal microarray analysis should provide genetic counselling to obtain informed consent, discuss the limitations of the methodology, obtain the parental decisions for return of incidental findings (II-2A) (recommendation 4) and provide post-test counselling for reporting of test results (III-A) (recommendation 9); (3) the resolution of chromosomal microarray analysis should be similar to postnatal microarray platforms to ensure small pathogenic variants are detected. To minimise the reporting of uncertain findings, it is recommended that variants of unknown significance (VOUS) smaller than 500 Kb deletion or 1 Mb duplication not be routinely reported in the prenatal context. Additionally, VOUS above these cut-offs should only be reported if there is significant supporting evidence that deletion or duplication of the region may be pathogenic (III-B) (recommendation 5); (4) secondary findings associated with a medically actionable disorder with

childhood onset should be reported, whereas variants associated with adult-onset conditions should not be reported unless requested by the parents or disclosure can prevent serious harm to family members (III-A) (recommendation 8). The working group recognises that there is variability across Canada in delivery of prenatal testing, and these recommendations were developed to promote consistency and provide a minimum standard for all provinces and territories across the country (recommendation 9).

BACKGROUND**Introduction**

Invasive prenatal diagnosis (PND) has been available since the 1970s and, since that time, has largely involved conventional karyotyping. Historically, PND was offered for primary investigation of possible fetal aneuploidy, with other structural chromosomal anomalies being detected simultaneously. Technological advances have increased the PND options to include quantitative fluorescence PCR (QF-PCR), which has now wholly replaced conventional cytogenetic analysis in some centres, for cases where the sole indication is increased risk of fetal aneuploidy of chromosomes 13, 18, 21, X or Y.¹ Whereas karyotyping is used in other specific higher-risk situations; this type of analysis has limitations, including limited genomic resolution and necessity of using cultured cells for analysis.

In addition to its ability to identify cases of aneuploidy, chromosomal microarray analysis (CMA) is a high-resolution technology capable of detecting microdeletions and microduplications throughout the genome. Depending on the methodology used, microarray analysis can detect additional concerns, including varying levels of mosaicism, cases of uniparental disomy (UPD) and possible consanguinity. For some time now, CMA has been used in the evaluation of children with unexplained developmental delay, autism spectrum disorder or congenital anomalies and has been shown to increase the diagnostic yield in such populations by up to 15% over conventional karyotyping.^{2,3} The improved resolution provided through CMA yields



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an increased rate of diagnosis of chromosome anomalies, as well as an increase in the rate of uncertain findings, unrelated findings or adult-onset disease indicators.

Canadian context

The delivery of healthcare services in Canada is largely regulated by the Canada Health Act that governs universal coverage for medically necessary healthcare services. However, which services are medically necessary is not defined in the Act; rather, it is up to each provincial and territorial government to determine the services that they will cover. Variation in provincial and territorial health programmes and policies results in significant inter-regional disparity among prenatal genetic screening and diagnostic programmes, despite established and emerging national guidelines. Further discrepancy is noted when evaluating service availability in rural versus urban contexts. In addition, individual physicians must balance the needs of their patients with the responsible use of finite resources within the system.

Given that access to prenatal testing is variable across Canada, this document is meant to provide some consistency and a minimum standard that should be available across the country. It is recognised that jurisdictions may choose to augment the minimum recommendations according to their own resource availability with due consideration of the implications of expanded testing. Therefore, it is strongly recommended that provincial/territorial standards be developed to ensure equal access across each region. Additionally, while access to services varies both among and within provinces, the recommendations that follow assume access to high quality ultrasound as a basic or fundamental minimum.

Methods

A multidisciplinary group consisting of medical geneticists, genetic counsellors, maternal fetal medicine specialists and clinical laboratory geneticists was assembled to review existing literature and guidelines for use of CMA in prenatal care and to make recommendations relevant to the Canadian context. The quality of evidence in this document was rated using modified criteria described in the Report of the Canadian Task Force on Preventive Healthcare,⁴ as used in the previous Society of Obstetricians and Gynaecologists of Canada (SOGC) guidelines.⁵ The statement was circulated for comment to the Canadian College of Medical Geneticists (CCMG) membership-at-large for feedback and, following incorporation of feedback, was approved by the CCMG Board of Directors (5 June 2017) and the SOGC Board of Directors (on 19 June 2017). The CCMG is a Canadian organisation responsible for certifying medical geneticists and clinical laboratory geneticists and for establishing professional and ethical standards for clinical genetics services in Canada. The SOGC is a Canadian organisation which produces national clinical guidelines for both public and medical, education and clinical practice, on important women's health issues. This guideline replaces the 2011 SOGC-CCMG Joint Technical Update.⁶

CLINICAL INDICATIONS

Given the complexity of interpretation and counselling, clinical prenatal chromosomal microarray analysis should only be ordered by a clinical geneticist or other physician with sufficient expertise in the use of the technology and the clinical interpretation of the results. In all instances, it is recommended that rapid aneuploidy detection (RAD) techniques be performed prior to chromosomal microarray analysis.

For samples obtained via invasive prenatal testing

In most jurisdictions, prenatal CMA is being employed in cases where multiple fetal anomalies have been detected by obstetric ultrasound.⁷⁻⁹ A 2011 joint SOGC-CCMG technical update concluded that prenatal CMA is advantageous in these situations.⁶

In instances with certain isolated fetal anomalies, prenatal CMA has been shown to provide additional diagnostic yield over that of conventional karyotyping and has also been recommended in such situations.⁷⁻¹⁰ Meta-analyses of CMA results in cases with isolated defects indicate a rate of pathogenic variants of ~5%.^{10,11} However, determining which individual malformations are most strongly associated with abnormal CMA results is challenging, as many papers report aggregate data by system, while others provide details of defects only in CMA positive cases. The systems with the highest yields of positive results in isolated cases are central nervous system (~6%), gastrointestinal (~7%), musculoskeletal (~8%) and cardiac (~7%).¹⁰⁻¹² Higher detection rates for pathogenic variants (>8%) have been observed with individual malformations including holoprosencephaly, cerebellar hypoplasia, hypoplastic left heart, cleft lip and/or palate and omphalocele.¹¹ In contrast, abnormal CMA results on fetuses with certain other isolated anomalies (eg, ventriculoseptal defect, gastroschisis, renal agenesis and lower urinary tract obstruction) do not appear to have been reported to date.

However, when a single structural defect is seen in association with other non-structural ultrasound findings, the frequency of pathogenic CMA results is higher, especially with intrauterine growth retardation or overgrowth (13.6%) or abnormal amniotic fluid volume (9.1%).¹¹

Increased nuchal translucency (NT) ≥ 3.5 mm, or >99 percentile, is associated with increased risk of fetal aneuploidy as well as increased risk of certain syndromes and other structural defects.¹³ A recent meta-analysis of the use of CMA in fetuses with increased NT and normal karyotype demonstrated incremental yields in diagnosis between 4% (in cases with isolated NT) and 7% (in cases with other malformations), with aberrations including those involving 22q11.2.¹⁴

In cases where an invasive procedure is undertaken for other reasons (eg, familial genetic conditions, positive prenatal screen, maternal age or anxiety), CMA is more frequently becoming an option after normal RAD results are available. This approach could be viewed as a form of opportunistic rather than diagnostic testing. As such, it may identify unrelated findings. A prospective study found that approximately 1.7% of pregnancies with late maternal age or a positive prenatal screen have a pathogenic or likely pathogenic copy number change.¹⁵ In any screening approach, the net benefit should be evident, healthcare providers with sufficient expertise should be available for appropriate follow-up and potential harms mitigated.¹⁶⁻¹⁸

Recommendation 1

- A. Offer of chromosomal microarray analysis (in addition to any other relevant diagnostic testing) is recommended in cases with multiple fetal anomalies identified by a comprehensive obstetric ultrasound (II-1A). Other diagnostic testing may include specific single gene, multigene panels or other genetic tests if the pattern of anomalies suggests a specific genetic condition not identified by array (II-2 A).
- B. Single structural defects in association with other abnormal ultrasound findings (eg, intrauterine growth restriction

- (IUGR), oligohydramnios) should not be considered isolated, and thus array should be offered if RAD is normal (III- B).
- C. In cases with a single fetal anomaly, prenatal CMA should be considered for those malformations associated with a high frequency of abnormal results. Its use in cases where the diagnostic yield is lower may be considered, if resources are available (III- B).
- D. In fetuses with an NT ≥ 3.5 mm, prenatal CMA should be offered (II-2 B).

The working group recognises that, in some instances, local resources allow for prenatal chromosomal microarray analysis to be available for all invasive prenatal specimens, outside of the above indications. While an approximate 1.7% risk of abnormal findings has been documented,¹⁵ there is also the impact of incidental findings and variants of unknown significance (VOUS) in a broad population (the so-called ‘toxic knowledge’) to be considered.¹⁹ At present, there is a paucity of evidence regarding the overall clinical utility and impact on outcomes associated with the broad use of CMA in routine prenatal diagnosis.²⁰ Therefore, local decision-makers must use caution and weigh the options and resources available prior to including CMA in the routine PND menu. In such situations, it is advised that this only be undertaken when there are concomitant clinical resources available to facilitate thorough pretest counselling and follow-up care.

For analysis of fetal loss prior to 20 weeks gestation

The majority of chromosome anomalies identified in early pregnancy loss are aneuploidies, detectable with conventional karyotyping.¹⁵ The 2010 CCMG guidelines recommended cytogenetic analysis of early pregnancy losses with evidence of growth retardation or congenital anomalies.²¹ Conventional cytogenetic analysis can be complicated by technical challenges that can result in low diagnostic yield from these non-viable pregnancies. Conversely, microarray can be performed directly on DNA from fetal samples, obviating the need for live, dividing cells and can provide a higher resolution analysis.^{22 23}

Recommendation 2

In cases of congenital anomalies and/or IUGR, in any fetal loss prior to 20 weeks gestation, if QF-PCR methodologies and/or other directed diagnostic inquiries do not provide a diagnosis and further cytogenetic analysis is intended, it is recommended that karyotype be replaced with chromosomal microarray analysis (III-B).

In the previous CCMG 2010 recommendation, parental karyotyping was recommended for couples after their third pregnancy loss.²¹ The working group noted that some jurisdictions may have the capacity to undertake CMA investigations for products of conception in these situations. Although there is little evidence to suggest that it has advantages over conventional cytogenetic analysis of the parents, CMA does have the potential to detect small unbalanced translocations in the fetus that may not be detectable by G-banding. Therefore the potential exists for higher diagnostic yield by using CMA to investigate the products of conception/fetus and should be considered further.

For samples from stillbirths, that is, fetal deaths at ≥ 20 weeks gestation

Evidence on the clinical utility of CMA in stillbirths is limited and is based on studies with wide-ranging criteria, methods and analysis. One area of clear advantage is with respect to failure rate associated with testing; unlike conventional karyotyping,

CMA does not require live cells, which allows for analysis of samples that would otherwise not be amenable to karyotype analysis.^{24 25} Some limitations of CMA include the inability to detect balanced translocations or polyploidy, and maternal contamination can still be a concern if extraembryonic tissue is analysed.²⁶ Interpretation of the significance of potentially pathogenic copy number variants (CNVs) can be problematic, especially if parental samples are not available.²⁷ When parental samples are available, few VOUS are found to be de novo events.²³

In stillbirths, aneuploidy is a more common finding (6%–7%) than pathogenic CNVs (~3%).^{27 28} Both are more frequent in stillbirths with structural malformations than in those without and in antepartum versus intrapartum fetal deaths.²³ Exact figures vary depending on whether array was done on all cases versus being done only if karyotyping failed or was normal. VOUS frequency is variable across subgroups; studies have reported ranges between 0.6%–2.1%²⁹ and 5%–6%²⁸ of samples.

Recommendation 3

- A. Aneuploidy is the most common abnormal chromosomal finding in stillbirths. If RAD and/or other directed diagnostic inquiries are uninformative, it is recommended that in cases complicated by congenital anomalies and/or IUGR, karyotype be replaced with CMA when further cytogenetic analysis is desired (II-2B).
- B. In stillbirths without structural fetal anomalies, CMA may be considered in the context of local resource availability and site-based postmortem protocol (whether complete, limited or external only) (II-2B).

PRETEST COUNSELLING

The various types and potential ambiguity of results available through prenatal CMA necessitates clear and thorough pretest counselling and consent. A professional with expertise and understanding of the complexity of CMA and the potential results, and who has the ability and time to provide unbiased information, should facilitate this counselling. For a list of specialty genetics centres across Canada, please refer to the Canadian Association of Genetic Counsellors website at www.cagc-accg.ca.

Discussion around approaches to incidental findings follows later in this document, but centres that choose to report such findings should ensure that patients receive counselling by qualified health professionals, focusing on both the risks and benefits of learning about this type of result. Counselling must include recognition of and discussion of possible adverse outcomes, including parents’ learning about secondary findings that they did not wish to know, such as identification of an adult onset disorder.

Recommendation 4

Given the varied contexts in which prenatal chromosomal microarray analysis may be offered, it is essential that pretest counselling be undertaken by a professional with expertise in the utilisation of CMA in the prenatal setting. The counselling content should be documented in the medical record. This pretest counselling should include:

- A. Formal informed consent for chromosomal microarray analysis, including the parental decisions regarding the receipt of secondary findings (subject to the limitations described in Recommendation 8), should be communicated clearly to the laboratory via the requisition to ensure that the

- report reflects only that information that was agreed to by the parents.
- B. Information regarding the limitations of the test methodology used,
 - C. Occurrence of variants of uncertain significance and the possibility of secondary findings.
 - D. Discussion of possible outcomes, including what will and what will not be reported, such as:
 - I. variants of uncertain significance;
 - II. CNVs associated with variable expressivity or penetrance;
 - III. secondary findings not related to the reason for testing;
 - IV. carrier identification, both for autosomal recessive and X-linked disorders.
 - E. Potential issues related to insurance and discrimination.
 - F. Potential need for parental samples and additional testing, accompanied by a discussion of what may be reported from parental samples.
 - G. Educational material that can be provided to supplement the clinical discussion to enable reflection beyond the clinic encounter.
 - H. If the family is concerned about the risk of a specific adult-onset condition, they should also be counselled regarding the pattern of inheritance and the appropriate testing options available to family members (III A).

CONSIDERATIONS FOR TECHNICAL ASPECTS AND REPORTING

CMA encompasses all types of array-based genomic copy number analyses, including array-based comparative genomic hybridisation and single nucleotide polymorphism (SNP) arrays. Both platforms can detect large scale and submicroscopic chromosomal imbalances (gains and losses) and mosaicism as low as approximately 10%–20%.^{30 31} The advantages of SNP arrays include the detection of copy neutral absence of heterozygosity (AOH), which may suggest UPD, identity by descent, parental consanguinity or loss of heterozygosity, as well as the detection of triploidy.^{32 33} Neither platform can identify balanced rearrangements.

In terms of testing methodology, direct testing on uncultured cells should be performed wherever possible. When performing analysis of chorionic villi, DNA should be isolated from the mesenchymal core cell fraction of uncultured chorionic villi. In ongoing pregnancies, protocols should be established (eg, establish reserve cell culture) to avoid the need to recollect a sample should initial DNA extraction fail to meet established quality standards, or should additional testing be required (eg, G-banding to resolve structural chromosome abnormalities). Maternal cell contamination should be investigated as per CCMG guideline: 'Recommendations for the Indications, Analysis and Reporting of Prenatal Specimens' (2010) (pending revision).

Copy number analysis

The working group agreed with the classification of CNVs recommended by the American College of Medical Genetics (ACMG):

- ▶ pathogenic;
- ▶ uncertain clinical significance: likely pathogenic;
- ▶ uncertain clinical significance;
- ▶ uncertain clinical significance: likely benign;
- ▶ benign.³⁴

Prenatal CMA should be capable of detecting CNVs with the same resolution as for postnatal CMA to ensure small '*pathogenic*' variants, or '*uncertain clinical significance: likely pathogenic*' variants will be detected and reported. It is recommended

that larger minimum threshold sizes for reporting variants of '*uncertain clinical significance*' should be applied for prenatal testing to decrease the reporting of such findings to parents, which can lead to significant parental anxiety.^{19 35} A 2014 Canadian microarray symposium reached consensus that for prenatal array, thresholds should be set at 500 kb for deletions and 1 Mb for duplications; CNVs of '*uncertain clinical significance*' that are smaller than these size limits should not be reported.³⁶ It was also agreed that variants of '*uncertain clinical significance*' exceeding these size thresholds should not be reported automatically; rather, such variants should only be reported if there is some published, but not necessarily conclusive, evidence that they may be pathogenic.

Recommendation 5

- A. CNVs should be interpreted by the laboratory after review of available literature and appropriate databases of benign and pathogenic variants (eg, ClinGen, ClinVar, Database of Genomic Variants, DECIPHER), including databases of missense, nonsense and in-del variants in the genes affected by the abnormality detected by CMA. This review should include consideration of the potential mechanism of morbidity of the impacted gene(s) (eg, loss or gain of function). Care should be taken in interpreting results where a parent originates from a population that is poorly represented in publicly available databases. Therefore, the lab should request parental ethnicity on the requisition.
- B. The resolution of analysis should be similar to that obtained from postnatal chromosomal microarray analysis³ to ensure that small variants that are classified as:
 - a. '*pathogenic*', or
 - b. '*uncertain clinical significance: likely pathogenic*' can be detected and reported (III-B).
- C. To minimise the reporting of uncertain findings, it is recommended that variants of '*uncertain clinical significance*' smaller than 500 Kb deletion or 1 Mb duplication not be routinely reported in the prenatal context. Additionally, such variants above these cut-offs should only be reported if there is some, but not necessarily conclusive, evidence that they may be pathogenic (III-B).
- D. Variants characterised as:
 - a. '*uncertain clinical significance: likely benign*' or;
 - b. '*benign*' should not be reported.
- E. When possible, parental CMA analysis or fluorescent in situ hybridisation/qPCR studies should also be performed to aid clinical interpretation, considering the possibility of reduced penetrance and variable expressivity.

Variable penetrance and expressivity

Many CNVs are associated with phenotypes that have reduced penetrance and/or variable expressivity, including autism spectrum disorder, psychiatric conditions and cognitive developmental disability.³⁷ Given that the predictive value is uncertain for the vast majority of such CNVs, other jurisdictions have narrowed the recommended reportable list of these susceptibility variants to those where the risk of neurodevelopmental disorders is established and where the CNV is associated with structural malformations that warrant additional ultrasound follow-up.^{9 38}

Recommendation 6

- A. The laboratory should report only those CNVs characterised by reduced penetrance/variable expressivity that have

multiple lines of evidence supporting a high risk of the deletion/duplication for neurodevelopmental abnormalities or congenital malformations. Examples of CNVs that fit these criteria have been published previously³⁸; however, the laboratory should apply the same principles as new CNVs meeting these criteria are identified.

- B. In the case of a female fetus found to be a carrier of a CNV on the X-chromosome, the report should indicate potential for phenotypic heterogeneity and the need for clinical interpretation.

Absence of heterozygosity

SNP arrays have the advantage of detecting long continuous stretches of homozygosity, which may reveal instances of UPD, identity by descent or loss of heterozygosity.^{32,39,40} The threshold for reporting AOH has been suggested to be greater than 10 to 13.5 MB.³⁹

Recommendation 7

- A. In cases with no identified consanguinity: (III-A)
- I. If a segment with AOH larger than 10 Mb is detected in one chromosome, and in the absence of genome-wide AOH (eg, total autosomal AOH <6%), then the possibility of UPD should be reported for chromosomes associated with an imprinting disorder (ie, 6, 7, 11, 14, 15).
 - II. If AOH is detected in a chromosome associated with a known UPD syndrome, definitive UPD testing is necessary.
 - III. As detection of AOH can be observed in the absence of UPD, and SNP arrays cannot detect all instances of UPD, it is recommended that the report include a disclaimer that SNP array is not a diagnostic assay for UPD.
- B. In cases with known consanguinity:
- I. AOH should not be reported unless specifically requested. However, in such instances, it is suggested that the report indicate that 'regions of AOH are available on request'.
- C. In all cases:
- I. If AOH results are consistent with a second degree or closer relationship between parents, the referring physician should be informed of this possibility to consider whether the mother may be at risk (eg, if the mother is a minor or a person with cognitive disability).
 - II. It is also important to indicate that a family history with multiple loops of consanguinity can increase the proportion of AOH across the genome of the proband and may overestimate the degree of relationship between parents. As such, it is recommended that the laboratory consults with the referring physician prior to including these results in the CMA report.

Approach to secondary findings

Prenatal chromosomal microarray analysis is employed specifically to correlate clinical indications with potential underlying chromosomal abnormalities, with the intent to inform immediate management of the pregnancy. The committee agrees prenatal chromosomal microarray analysis should not specifically search for CNVs unrelated to the primary reason for referral. Similarly, the CCMG guideline for genome-wide sequencing recommends a cautious approach to reporting secondary findings, which includes the avoidance of analysing genes unrelated to the primary diagnosis.⁴¹ However, the nature of chromosomal microarray analysis is such that CNVs often

include many genes and may include those related to secondary or incidental findings associated with adult-onset conditions or carrier status.

Other jurisdictions have reached differing conclusions regarding reporting of secondary findings. In the UK, unsolicited pathogenic findings are recommended to be reported only when the identified variant may inform present or future management of the pregnancy or family; non-actionable findings are not to be reported.⁹ When considering incidental findings in clinical exome and whole genome sequencing, the ACMG has allowed patients to opt out of such results, requiring discussion at the time of patient consent, prior to sample submission.⁴²

The working group recognises that there is limited evidence available with regard to the benefits, risks and costs of disclosing secondary findings arising from prenatal CMA and that the reporting of secondary findings is a controversial issue. For secondary findings that have been identified during the course of prenatal chromosomal microarray analysis, the working group used a similar rationale to the CCMG guideline for genome-wide sequencing.⁴¹ Since all scenarios identified by the working group for prenatal CMA were not addressed in the CCMG guideline for genome-wide sequencing, the recommendations were based on the available evidence and group consensus.

Recommendation 8: (III-B)

- A. Secondary findings that reveal risk for a highly penetrant condition that is medically actionable during childhood should be reported.
- B. It is recommended that laboratories not report fetal risk for adult-onset conditions unless
- I. the parents specifically request this information, or
 - II. disclosure of the information could prevent serious harm to the health of other family members (eg, pathogenic mutation in a gene with high risk of cancer susceptibility).
- C. It is recommended that laboratories NOT report non-actionable secondary findings (eg, related to early onset Alzheimer's disease).
- D. The laboratory does not have the obligation to later re-contact patients to advise of potential adult-onset conditions if they were not included in the prenatal array report.
- E. It is not recommended that the analysis seek to identify CNVs associated with carrier status for autosomal recessive disorders. However, if in the course of analysis such information is identified, carrier status of autosomal recessive disease with a high carrier frequency in the population being tested (eg, $\geq 1/50$ as has been suggested by other jurisdictions³⁸ or as per local policy) should be reported.
- F. It is not recommended that the analysis seek to identify CNVs associated with X-linked recessive carrier status. However, if in the course of analysis such information is identified, the laboratory should report female carriers of X-linked recessive mutations associated with childhood-onset disorders, since there may be significant risk to the family for conceiving affected males.

It is important for each centre to have a clear policy for each consideration above to facilitate transparent practices and patient counselling.

POST-TEST COUNSELLING

As with pretest counselling, prenatal CMA results should be provided to parents by a professional with thorough understanding of and experience with microarray analysis. Even in the context of well-described conditions, there can remain

ambiguity due to phenotype variability that cannot be clarified in the prenatal setting.¹⁹

All CMA results should be interpreted in the particular clinical context in which the analysis was undertaken. The professional communicating results should discuss that a negative result does not guarantee a normal postnatal outcome, reviewing testing limitations as was done in the pretest counselling. Regardless of the potential severity of the returned results, involvement of a specialist genetics service in the interpretation, counselling and follow-up of prenatal CMA is recommended.

Recommendation 9: (III-A)

- A. As with conventional karyotyping or other targeted diagnostic testing, the possibility of other genetic anomalies that are not detectable by CMA should be discussed.
- B. Any CNVs reported to parents should be in the context of appropriate genetic counselling, incorporating parental results and any additional information available.
- C. Irrevocable obstetrical decisions due to CMA findings should not be made without referring to a genetics specialty service,^{19 43} unless the decisions are based on the presence of malformations or other pregnancy concerns. The resources and expertise for multidisciplinary discussion and counselling, including ethics consultation, may be required in some cases.
- D. Special precautions should be taken in counselling families who have educational, linguistic and/or cultural barriers to a full understanding of the counselling and who may have different prior assumptions about the predictive value of genetic testing results (sometimes referred to as ‘genetic determinism’).

CONCLUSION

This practice guideline serves to inform Canadian providers with direction regarding the use of chromosomal microarray analysis in the prenatal setting across Canada. It was developed based on current evidence and will evolve as further evidence arises.

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REFERENCES

- 1 Langlois S, Duncan A. SOGC Genetics Committee, CCMG Prenatal Diagnosis Committee. Use of a DNA method, QF-PCR, in the prenatal diagnosis of fetal aneuploidies. *J Obstet Gynaecol Can* 2011;33:955–60.
- 2 Miller DT, Adam MP, Aradhya S, Biesecker LG, Brothman AR, Carter NP, Church DM, Crolla JA, Eichler EE, Epstein CJ, Faucett WA, Feuk L, Friedman JM, Hamosh A, Jackson L, Kaminsky EB, Kok K, Krantz ID, Kuhn RM, Lee C, Ostell JM, Rosenberg C, Scherer SW, Spinner NB, Stavropoulos DJ, Tepperberg JH, Thorland EC, Vermeesch JR, Waggoner DJ, Watson MS, Martin CL, Ledbetter DH. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *Am J Hum Genet* 2010;86:749–64.
- 3 Stavropoulos DJ, Shago M. Genomic microarray testing – CCMG guideline. 2016 https://www.ccmg-ccgm.org/images/job_postngs/CCMG_Guidelines_for_Genomic_Microarray_Testing_FINAL.pdf (accessed 1 May 2017).
- 4 Woolf S, Battista R, Angerson G, Logan A, Eel W; Canadian Task Force on Preventive Health Care. New grades for recommendations from the Canadian Task Force on Preventive Health Care. *CMAJ* 2003;169:207–8.
- 5 Wilson RD, De Bie I, Armour CM, Brown RN, Campagnolo C, Carroll JC, Okun N, Nelson T, Zwingerman R, Audibert F, Brock JA, Brown RN, Campagnolo C, Carroll JC, De Bie I, Johnson JA, Okun N, Pastruck M, Vallée-Pouliot K, Wilson RD, Zwingerman R, Armour C, Chitayat D, De Bie I, Fernandez S, Kim R, Lavoie J, Leonard N, Nelson T, Taylor S, Van Allen M, Van Karnebeek C. Joint SOGC-CCMG opinion for reproductive genetic carrier screening: an update for all Canadian providers of maternity and reproductive healthcare in the era of direct-to-consumer testing. *J Obstet Gynaecol Can* 2016;38:742–62.
- 6 Duncan A, Langlois S; SOGC Genetics Committee, CCMG Prenatal Diagnosis Committee. Use of array genomic hybridization technology in prenatal diagnosis in Canada. *J Obstet Gynaecol Can* 2011;33:1256–9 <http://www.ncbi.nlm.nih.gov/pubmed/22166281>.
- 7 Hillman S, McMullan DJ, Maher ER, Kilby MD. The use of chromosomal microarray in prenatal diagnosis. *Obstet Gynaecol* 2013;15:80–4.
- 8 Novelli A, Grati FR, Ballarati L, Bernardini L, Bizzoco D, Camurri L, Casalone R, Cardarelli L, Cavalli P, Ciccone R, Clementi M, Dalprà L, Gentile M, Gelli G, Grammatico P, Malacarne M, Nardone AM, Pecile V, Simoni G, Zuffardi O, Giardino D. Microarray application in prenatal diagnosis: a position statement from the cytogenetics working group of the Italian Society of Human Genetics (SIGU), November 2011. *Ultrasound Obstet Gynecol* 2012;39:384–8.
- 9 Gardiner C, Wellesley D, Kilby MD, Kerr B. Recommendations for the use of chromosome microarray in pregnancy. 2015;2015:1–17 <https://www.rcpath.org/resourceLibrary/recommendations-for-the-use-of-chromosome-microarray-in-pregnancy.html>
- 10 de Wit MC, Srebniak MI, Govaerts LC, Van Opstal D, Galjaard RJ, Go AT. Additional value of prenatal genomic array testing in fetuses with isolated structural ultrasound

- abnormalities and a normal karyotype: a systematic review of the literature. *Ultrasound Obstet Gynecol* 2014;43:139–46.
- 11 Shaffer LG, Rosenfeld JA, Dabell MP, Coppinger J, Bandholz AM, Ellison JW, Ravnar JB, Torchia BS, Ballif BC, Fisher AJ. Detection rates of clinically significant genomic alterations by microarray analysis for specific anomalies detected by ultrasound. *Prenat Diagn* 2012;32:986–95.
 - 12 Jansen FA, Blumenfeld YJ, Fisher A, Cobben JM, Odibo AO, Borrell A, Haak MC. Array comparative genomic hybridization and fetal congenital heart defects: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 2015;45:27–35.
 - 13 Ayräs O, Tikkanen M, Eronen M, Paavonen J, Stefanovic V. Increased nuchal translucency and pregnancy outcome: a retrospective study of 1063 consecutive singleton pregnancies in a single referral institution. *Prenat Diagn* 2013;17.
 - 14 Grande M, Jansen FA, Blumenfeld YJ, Fisher A, Odibo AO, Haak MC, Borrell A. Genomic microarray in fetuses with increased nuchal translucency and normal karyotype: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 2015;46:650–8.
 - 15 Wapner RJ, Martin CL, Levy B, Ballif BC, Eng CM, Zachary JM, Savage M, Platt LD, Saltzman D, Grobman WA, Klugman S, Scholl T, Simpson JL, McCall K, Aggarwal VS, Bunke B, Nahum O, Patel A, Lamb AN, Thom EA, Beaudet AL, Ledbetter DH, Shaffer LG, Jackson L. Chromosomal microarray versus karyotyping for prenatal diagnosis. *N Engl J Med* 2012;367:2175–84.
 - 16 Wilson J, Jungner G. Principles and practice of screening for disease. *Arch Intern Med* 1969;123:349.
 - 17 Harris R. Overview of screening: where we are and where we may be headed. *Epidemiol Rev* 2011;33:1–6.
 - 18 Grosse SD, Rogowski WH, Ross LF, Cornel MC, Dondorp WJ, Khoury MJ. Population screening for genetic disorders in the 21st century: evidence, economics, and ethics. *Public Health Genomics* 2010;13:106–15.
 - 19 Bernhardt BA, Soucier D, Hanson K, Savage MS, Jackson L, Wapner RJ. Women's experiences receiving abnormal prenatal chromosomal microarray testing results. *Genet Med* 2013;15:139–45.
 - 20 Callaway JL, Shaffer LG, Chitty LS, Rosenfeld JA, Crolla JA. The clinical utility of microarray technologies applied to prenatal cytogenetics in the presence of a normal conventional karyotype: a review of the literature. *Prenat Diagn* 2013;33:1119–23.
 - 21 CCMG. CCMG Practice Guidelines for Cytogenetic Analysis B. *Recommendations for the indications, analysis and reporting of constitutional specimens (peripheral blood, solid tissues)* Prepared: CCMG Cytogenetics Committee Approved by CCMG Bo, 2010.
 - 22 Levy B, Sigurjonsson S, Pettersen B, Maisenbacher MK, Hall MP, Demko Z, Lathi RB, Tao R, Aggarwal V, Rabinowitz M. Genomic imbalance in products of conception. *Obstet Gynecol* 2014;124:202–9.
 - 23 Rosenfeld JA, Tucker ME, Escobar LF, Neill NJ, Torchia BS, McDaniel LD, Schultz RA, Chong K, Chitayat D. Diagnostic utility of microarray testing in pregnancy loss. *Ultrasound Obstet Gynecol* 2015;46:478–86.
 - 24 Reddy UM, Page GP, Saade GR. The role of DNA microarrays in the evaluation of fetal death. *Prenat Diagn* 2012;32:371–5.
 - 25 Raca G, Artzer A, Thorson L, Huber S, Modaff P, Laffin J, Pauli RM. Array-based comparative genomic hybridization (aCGH) in the genetic evaluation of stillbirth. *Am J Med Genet A* 2009;149A:2437–43.
 - 26 Menten B, Swerts K, Delle Chiaie B, Janssens S, Buysse K, Philippé J, Speleman F. Array comparative genomic hybridization and flow cytometry analysis of spontaneous abortions and mors in utero samples. *BMC Med Genet* 2009;10:89.
 - 27 Sahlin E, Gustavsson P, Liedén A, Papadogiannakis N, Bjärebörn L, Pettersson K, Nordenskjöld M, Iwarsson E. Molecular and cytogenetic analysis in stillbirth: results from 481 consecutive cases. *Fetal Diagn Ther* 2014;36:326–32.
 - 28 Reddy UM, Page GP, Saade GR, Silver RM, Thorsten VR, Parker CB, Pinar H, Willinger M, Stoll BJ, Heim-Hall J, Varner MW, Goldenberg RL, Bukowski R, Wapner RJ, Drews-Botsch CD, O'Brien BM, Dudley DJ, Levy B; NICHD Stillbirth Collaborative Research Network. Karyotype versus microarray testing for genetic abnormalities after stillbirth. *N Engl J Med* 2012;367:2185–93.
 - 29 Wou K, Hyun Y, Chitayat D, Vlasschaert M, Chong K, Wasim S, Keating S, Shannon P, Kolomietz E. Analysis of tissue from products of conception and perinatal losses using QF-PCR and microarray: a three-year retrospective study resulting in an efficient protocol. *Eur J Med Genet* 2016;1–8.
 - 30 Ballif BC, Rorem EA, Sundin K, Lincicum M, Gaskin S, Coppinger J, Kashork CD, Shaffer LG, Bejjani BA. Detection of low-level mosaicism by array CGH in routine diagnostic specimens. *Am J Med Genet A* 2006;140:2757–67.
 - 31 Cross J, Peters G, Wu Z, Brohede J, Hannan GN. Resolution of trisomic mosaicism in prenatal diagnosis: estimated performance of a 50K SNP microarray. *Prenat Diagn* 2007;27:1197–204.
 - 32 Kearney HM, Kearney JB, Conlin LK. Diagnostic implications of excessive homozygosity detected by SNP-based microarrays: consanguinity, uniparental disomy, and recessive single-gene mutations. *Clin Lab Med* 2011;31:595–613.
 - 33 Conlin LK, Thiel BD, Bonnemann CG, Medne L, Ernst LM, Zackai EH, Deardorff MA, Krantz ID, Hakonarson H, Spinner NB. Mechanisms of mosaicism, chimerism and uniparental disomy identified by single nucleotide polymorphism array analysis. *Hum Mol Genet* 2010;19:1263–75.
 - 34 Kearney HM, Thorland EC, Brown KK, Quintero-Rivera F, South ST; Working Group of the American College of Medical Genetics Laboratory Quality Assurance Committee. American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants. *Genet Med* 2011;13:680–5.
 - 35 Bernhardt BA, Kellom K, Barbarese A, Faucett WA, Wapner RJ. An exploration of genetic counselors' needs and experiences with prenatal chromosomal microarray testing. *J Genet Couns* 2014;23:938–47.
 - 36 Buchanan JA, Chitayat D, Kolomietz E, Lee HC, Scherer SW, Speevak MD, Sroka H, Stavropoulos DJ. Prenatal genomic microarray and sequencing in Canadian medical practice: towards consensus. *J Med Genet* 2015;52:585–6.
 - 37 Rosenfeld JA, Coe BP, Eichler EE, Cuckle H, Shaffer LG. Estimates of penetrance for recurrent pathogenic copy-number variations. *Genet Med* 2013;15:478–81.
 - 38 Vanakker O, Vilain C, Janssens K, Van der Aa N, Smits G, Bandelier C, Blaumeiser B, Bulk S, Caberg JH, De Leener A, De Rademaeker M, de Ravel T, Desir J, Destree A, Dheedene A, Gaillez S, Grisart B, Hellin AC, Janssens S, Keymolen K, Menten B, Pichon B, Ravoot M, Revencu N, Rombout S, Staessens C, Van Den Bogaert A, Van Den Bogaert K, Vermeesch JR, Kooy F, Sznajer Y, Devriendt K. Implementation of genomic arrays in prenatal diagnosis: the Belgian approach to meet the challenges. *Eur J Med Genet* 2014;57:151–6.
 - 39 Papehausen P, Schwartz S, Rishag H, Keitges E, Gadi I, Burnside RD, Jaswaney V, Pappas J, Pasion R, Friedman K, Tepperberg J. UPD detection using homozygosity profiling with a SNP genotyping microarray. *Am J Med Genet A* 2011;155A:757–68.
 - 40 Bruno DL, White SM, Ganesamoorthy D, Burgess T, Butler K, Corrie S, Francis D, Hills L, Prabhakara K, Ngo C, Norris F, Oertel R, Pertile MD, Stark Z, Amor DJ, Slater HR. Pathogenic aberrations revealed exclusively by single nucleotide polymorphism (SNP) genotyping data in 5000 samples tested by molecular karyotyping. *J Med Genet* 2011;48:831–9.
 - 41 Boycott K, Hartley T, Adam S, Bernier F, Chong K, Fernandez BA, Friedman JM, Geraghty MT, Hume S, Knoppers BM, Laberge AM, Majewski J, Mendoza-Londono R, Meyn MS, Michaud JL, Nelson TN, Richer J, Sadikovic B, Skidmore DL, Stockley T, Taylor S, van Karnebeek C, Zawati MH, Lauzon J, Armour CM; Canadian College of Medical Geneticists. The clinical application of genome-wide sequencing for monogenic diseases in Canada: position statement of the Canadian College of Medical Geneticists. *J Med Genet* 2015;52:431–7.
 - 42 ACMG Board of Directors. ACMG policy statement: updated recommendations regarding analysis and reporting of secondary findings in clinical genome-scale sequencing. *Genet Med* 2015;17:68–9.
 - 43 Dondorp WJ, Sikkema-Raddatz B, de Die-Smulders C, de Wert G. Arrays in postnatal and prenatal diagnosis: an exploration of the ethics of consent. *Hum Mutat* 2012;33:916–22.