CEOG Clinical and Experimental Obstetrics & Gynecology

Review Article

Chromosomal microarray analysis in prenatal diagnosis

Yingjun Xie, Xiaofang Sun

Key Laboratory for Major Obstetric Diseases of Guangdong Province, Key Laboratory of Reproduction and Genetics of Guangdong Higher Education Institutes, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou (China)

Summary

Genome copy number variation (CNV) is an important cause of genetic and developmental disorders. In recent years, chromosomal microarray analysis (CMA) technology to test for genomic copy number variation has been developed and gradually applied in prenatal diagnostics, offering high diagnostic ability. Here, the authors summarise the CMA established in clinical settings, as well as the significance and clinical application of the standard analyses. They also emphatically discuss the key problems in the establishment process of the platform in prenatal diagnostics in the clinic.

Key words: Genome copy number variation; Chromosomal microarray analysis; Prenatal diagnosis; Detection platform.

Introduction

Genome copy number variation (CNV) (deletion/duplication) is widely distributed in the whole human genome [1]. It is closely connected with the emergence of new technologies, including high resolution methods of microarray and next-generation sequencing. Obviously, large-scale structural changes profoundly influence genetic variation. By various molecular mechanisms, including gene dosage, gene disruption, gene fusion, and position effects, CNVs can cause Mendelian or sporadic traits, or be associated with complex diseases [2, 3]. It is also clear that many gene CNVs produce deleterious phenotypic consequences. Particularly, de novo gene CNV is one of the important causes of genetic and developmental disorders including severe mental disabilities, autism, schizophrenia, and heart defects, and is frequently found in cancer cells [2, 4-6].

CNV detection method

In the past 40 years, chromosome analysis using G banding has been considered the gold standard for detection of chromosomal abnormalities. However, this method is time-consuming, requires cell culture, has limited resolution, and is not sufficient for the detection of less than five MB of chromosomal abnormalities. To further identify changes less than five MB in size, a combination of genetics and molecular biology methods was developed. Fluorescence in situ hybridisation technique (FISH) and multiplex ligation-dependent probe amplification (MLPA) can detect chromosome imbalances smaller than one MB, but these techniques can only be used in a limited area of chromosomal abnormalities, and a pre-test must be expected for the detection of disease and targeted. In prenatal diagnosis, chromosomal microarray analysis (CMA) can detect genome level abnormalities, and no cell culture is required, thus shortening the time that patients await results that can be used for the detection of fetal death or stillbirth. Additionally, CMA is a standardised process that utilises computer analysis, whereas karyotyping requires microscopic examination of chromosomes after staining. Karyotype analysis may be more subjective and prone to human error.

CMA technology, also known as molecular karyotype analysis technique (molecular karyotyping), is based on microarray genome copy number analysis technologies including comparative genomic hybridisation array (aCGH) technology and single nucleotide polymorphism microarray (SNP array) technology. CMA can detect unbalanced chromosome CNV at the entire genome level and can be used to identify chromosomal abnormalities, including those that are too small to detect by conventional karyotype analysis, tiny chromosomal abnormalities. The probe design, the original data acquisition, the data analysis algorithms, and other factors are the key causes to inconsistent results, and the CMA detection technology in clinical diagnostics using quality requirements are presented here along with the corresponding goals [7].

CMA clinical application of the status quo

CMA is currently the most effective tool to evaluate copy number abnormalities, which are abnormalities due to large

Revised manuscript accepted for publication July 15, 2015

Clin. Exp. Obstet. Gynecol. - ISSN: 0390-6663
XLIV, n. 2, 2017
doi: 10.12891/ceog3218.2017

7847050 Canada Inc.
www.irog.net

spanning length CNVs that can contain a part of a gene or genes and can span multiple genes [8, 9]. CMA can detect the total number of nucleotides in the genome more effectively than single nucleotide polymorphisms (SNP) [10-13]. CNV formation occurs by both recombination-based and replication-based mechanisms, and de novo locus-specific mutation rates are much higher for CNVs than for SNPs. The locus-specific mutation rates for CNVs have been observed to be ~100 to 10,000 times higher than those for nucleotide substitution rates [2, 14]. Additionally, postpartum data from multiple centres indicated that the overall risk of not diagnosing a microdeletion or microduplication syndrome associated with a CNV is higher than for chromosomal or single gene disease, thus contributing to a relatively high risk of mental retardation and abnormal growth and development [12, 15-17].

Over the past ten years, the efficiency of CMA has been widely recognised. CMA improves the detection level of known diseases, as well as syndromes that are caused or associated with unknown chromosome micro-deletions/duplications, promoting a diagnostic accuracy in clinical settings. When CMA was first implemented as a clinical application, many scholars suspected that CMA would likely replace the existing cytogenetic analysis method. However, CMA can only detect abnormal genome copy number changes relative to a reference genome, but does not enable the detection of balanced translocations, inversions, or inverted insertions, low levels of mosaicism, gene rearrangements or point mutations. Furthermore, CNVs in the human genome contribute to both Mendelian and complex traits, as well as to genomic plasticity in evolution, and especially to common and complex diseases [18]. Therefore, CMA, as well as other techniques, would be important in our clinical platform for the detection of various diseases caused by genomic rearrangement. In addition, a recent study showed that non-invasive prenatal testing (NIPT) using microarray analysis delivered a more accurate cell-free DNA (cfDNA) analysis than next-generation sequencing (NGS), and can be performed in less time [19].

The establishment of the CMA testing platform

Prenatal CMA requires professional genetic counselling before and after the test results are presented to the patients, so that the patients are aware of the benefits and limitations of the testing such that they can independently make choices regarding their pregnancy based on a full understanding of the benefits, limitations, and the results of CMA testing [10, 15, 20, 21]. Based on data from multiple prenatal diagnosis centres, it is apparent that variant of unknown significance (VOUS) is inevitably best as an antenatal examination, although it is not difficult to understand the status quo from the aspects of the complexity of human genome diversity and restructuring. However, this undoubtedly increases the complexity of clinical genetic counselling, and represents the main limitation of CMA in the clinical application of prenatal diagnosis [13, 22]. Through continuous accumulation of experience, test results can be increasingly clearly identified as benign or pathological. However, in prenatal diagnosis, due to the instability of expression and incomplete penetrance, many test results remain poorly understood [13]. The comparison between the large sample size case group and the control group can establish each CNV pathogenic spectrum, allowing for the minimisation of VOUS; it is only a matter of time before VOUS is mediated [23]. Furthermore, the satisfaction of patient genetic counselling is associated with the experience of genetic counsellors with CMA, the high enthusiasm of genetic counsellors for CMA, and additional CMA professional training [24, 25].

Assessing the clinical relevance of copy number variation, as a clinical test, should follow compliance-based medicine (evidence-based medicine, EBM) rules; that is, that medical decisions should be based on the best scientific evidence attainable [17, 26]. The sources of evidence, including clinical case reports, case series, and case-control analyses, among others, should be added to and supplemented by a public database that can be shared, such as DECIPHER, DGVs, ISCA, OMIM, *et al.* [27-29]. However, these data should be used with caution and common sense: the quality of these databases, inconsistent reference populations, poor annotation of genomic and phenotypic data, a lack of data curation, and inconsistent descriptions of the phenotype variation all may affect the effective application and clinical interpretation of the CMA results [30]. In addition the testing results of different technical platforms for the inconsistencies in probe design, original data acquisition, and data analysis algorithms can lead to inconsistent results, and use of CMA technology for clinical diagnostics requires corresponding quality requirements [7]. However, most of these problems will be solved in line with the development of concern regarding them [31-33].

In summary, CMA testing technology is of realistic significance in clinical practice and can be applied to large patient cohorts. The diagnostic implementation of NGS technologies creates new possibilities for the simultaneous testing of single nucleotide variations (SNVs), indels, and CNVs [34]. These technologies will further improve, if this detection is combined with whole exome and genome sequencing. However, the effective and confident clinical interpretation of the extensive quantity and variety of data information will remain a considerable challenge.

Acknowledgements

This work is supported by the NSFC (31171229, U1132005); Science and Technology of Guangzhou KeyProject (GZ2011Y1-00038\20140000000-4,3); Guangdong Province (2013B051000087).

References

[1] Redon R., Ishikawa S., Fitch K.R., Feuk L., Perry G.H., Andrews T.D., *et al.*: "Global variation in copy number in the human genome". *Nature*, 2006, *444*, 444.
[2] Zhang F., Gu W., Hurles M.E., Lupski J.R.: "Copy number variation in human health, disease, and evolution". *Annu Rev. Genomics Hum. Genet.*, 2009, *10*, 451.
[3] Piotrowski A., Bruder C.E., Andersson R., Diaz de Stahl T., Menzel U., Sandgren J., *et al.*: "Somatic mosaicism for copy number variation in differentiated human tissues". *Hum. Mutat.,* 2008, *29*, 1118.
[4] Shelling A.N., Ferguson L.R.: "Genetic variation in human disease and a new role for copy number variants". *Mutat. Res.*, 2007, *622*, 33.
[5] Wain L.V., Armour J.A., Tobin M.D.: "Genomic copy number variation, human health, and disease". *Lancet*, 2009, *374*, 340.
[6] Yeo R.A., Gangestad S.W., Gasparovic C., Liu J., Calhoun V.D., Thoma RJ, *et al.*: "Rare copy number deletions predict individual variation in human brain metabolite concentrations in individuals with alcohol use disorders". *Biol. Psychiatry,* 2011, *70*, 537.
[7] Shen Y., An Y., Wu B.L.: "Standardization and diversification of copy number microarray testing for clinical diagnostics--implications of the cross-platform/algorithm study on clinical diagnostic chromosomal microarray analysis". *Clin. Chem.*, 2011, *57*, 1354.
[8] Hillman S.C., McMullan D.J., Hall G., Togneri F.S., James N., Maher E.J., *et al.*: "Use of prenatal chromosomal microarray: prospective cohort study and systematic review and meta-analysis". *Ultrasound Obstet. Gynecol.*, 2013, *41*, 610.
[9] Kang J.U., Koo S.H.: "Clinical implementation of chromosomal microarray technology in prenatal diagnosis. (Review)". *Mol. Med. Rep.,* 2012, *6*, 1219.
[10] American College of Obstetricians and Gynecologists Committee on Genetics: "Committee Opinion No. 581: the use of chromosomal microarray analysis in prenatal diagnosis". *Obstet. Gynecol.*, 2013, *122*, 1374.
[11] Guo X., Yanna, Ma X., An J., Shang Y., Huang Q., *et al.*: "A meta-analysis of array-CGH studies implicates antiviral immunity pathways in the development of hepatocellular carcinoma". *PloS One*, 2011, *6*, e28404.
[12] Klugman S., Suskin B., Spencer B.L., Dar P., Bajaj K., Powers J., *et al.*: "Clinical utility of chromosomal microarray analysis in prenatal diagnosis: report of first 6 months in clinical practice". *J. Matern. Fetal Neonatal Med.,* 2014, *27*, 1333.
[13] Zilina O., Teek R., Tammur P., Kuuse K., Yakoreva M., Vaidla E., *et al.*: "Chromosomal microarray analysis as a first-tier clinical diagnostic test: Estonian experience". *Mol. Genet. Genomic Med.,* 2014, *2*, 166.
[14] Alesi V., Bertoli M., Sinibaldi L., Novelli A.: "The clinical utility and indications of chromosomal microarray analysis in prenatal diagnosis". *BJOG,* 2013, *120*, 119.
[15] Lee C.N., Lin S.Y., Shih J.C., Su Y.N.: "Authors' response to: the clinical utility and indications of chromosomal microarray analysis in prenatal diagnosis". *BJOG,* 2013, *120*, 120.
[16] Mademont-Soler I., Morales C., Soler A., Martinez-Crespo J.M., Shen Y., Margarit E., *et al.*: "Prenatal diagnosis of chromosomal abnormalities in fetuses with abnormal cardiac ultrasound findings: evaluation of chromosomal microarray-based analysis". *Ultrasound Obstet. Gynecol.,* 2013, *41*, 375.
[17] Michelson D.J., Shevell M.I., Sherr E.H., Moeschler J.B., Gropman A.L., Ashwal S.: "Evidence report: genetic and metabolic testing on children with global developmental delay: report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society". *Neurology,* 2011, *77*, 1629.
[18] Fu W, Zhang F, Wang Y, Gu X, Jin L: Identification of copy number variation hotspots in human populations. *Am J Hum Genet.,* 2010, 87(4):494-504.
[19] Juneau K., Bogard P.E., Huang S., Mohseni M., Wang E.T., Ryvkin P., *et al.*: "Microarray-based cell-free DNA analysis improves noninvasive prenatal testing". *Fetal Diagn. Ther.*, 2014, *36*, 282.
[20] Armengol L., Nevado J., Serra-Juhe C., Plaja A., Mediano C., Garcia-Santiago F.A., *et al.*: "Clinical utility of chromosomal microarray analysis in invasive prenatal diagnosis". *Hum. Genet.,* 2012, *131*, 513.
[21] Kashork C.D., Theisen A., Shaffer L.G.: "Prenatal diagnosis using array CGH". *Methods Mol. Biol.*, 2008, *444*, 59.
[22] de Jong A., Dondorp W.J., Macville M.V., de Die-Smulders C.E., van Lith J.M., de Wert G.M.: "Microarrays as a diagnostic tool in prenatal screening strategies: ethical reflection". *Hum. Genet.,* 2014, *133*, 163.
[23] Mikhaelian M., Veach P.M., MacFarlane I., LeRoy B.S., Bower M.: "Prenatal chromosomal microarray analysis: a survey of prenatal genetic counselors' experiences and attitudes". *Prenat Diagn.,* 2013, *33*, 371.
[24] Redlinger-Grosse K.: "It is time: a commentary on "An exploration of genetic counselors' needs and experiences with prenatal chromosomal microarray testing". *J. Genet. Couns.*, 2014, *23*, 933.
[25] Bernhardt B.A., Kellom K., Barbarese A., Faucett W.A., Wapner R.J.: "An exploration of genetic counselors' needs and experiences with prenatal chromosomal microarray testing". *J. Genet. Couns.*, 2014, *23*, 938.
[26] Riggs E.R., Church D.M., Hanson K., Horner V.L., Kaminsky E.B., Kuhn R.M., *et al.*: "Towards an evidence-based process for the clinical interpretation of copy number variation". *Clin. Genet.,* 2012, *81*, 403.
[27] Mkrtchyan H., Gross M., Hinreiner S., Polytiko A., Manvelyan M., Mrasek K., *et al.*: "The human genome puzzle - the role of copy number variation in somatic mosaicism". *Curr. Genomics*, 2010, *11*, 426.
[28] Tchinda J., Lee C.: "Detecting copy number variation in the human genome using comparative genomic hybridization". *Biotechniques*, 2006, *41*, 385.
[29] Zhao M., Zhao Z.: "CNVannotator: a comprehensive annotation server for copy number variation in the human genome". *PloS One*, 2013, *8*, e80170.
[30] Hehir-Kwa J.Y., Wieskamp N., Webber C., Pfundt R., Brunner H.G., Gilissen C., *et al.*: "Accurate distinction of pathogenic from benign CNVs in mental retardation". *PLoS Comput. Biol.*, 2010, *6*, e1000752.
[31] Flachsbart F., Caliebe A., Heinsen F.A., Hemming-Karlsen T., Schreiber S., Franke A., Nebel A.: "Investigation of complement component C4 copy number variation in human longevity". *PloS One*, 2014, *9*, e86188.
[32] Hollox E.J., Hoh B.P.: "Human gene copy number variation and infectious disease". *Hum. Genet.,* 2014, *133*, 1217.
[33] Wakui K.: "Study of structural chromosome abnormalities to increase the understanding of human genetic diversity: a commentary on signature of backward replication slippage at the copy number variation junction". *J Hum. Genet.,* 2014, *59*, 591.
[34] Hehir-Kwa J.Y., Pfundt R., Veltman J.A., de Leeuw N.: "Pathogenic or not? Assessing the clinical relevance of copy number variants". *Clin. Genet.*, 2013, *84*, 415.

Corresponding Author:
XIAOFANG SUN, M.D.
The Third Affiliated Hospital of
Guangzhou Medical University
63 Duobao Rd.
Guangzhou 510150 (China)
e-mail: xiaofangsun@hotmail.com