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Original Article

The application of chromosomal microarray analysis to the prenatal diagnosis of isolated mild ventriculomegaly

Hong-Lei Duan, Xiang-Yu Zhu, Yu-Jie Zhu, Xing Wu, Guang-Feng Zhao, Wan-Jun Wang, Jie Li*

Department of Obstetrics and Gynecology, Affiliated Drum Tower Hospital, Medical School of Nanjing University, Nanjing, China

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ABSTRACT

Objective: To investigate the clinical value of chromosomal microarray analysis (CMA) in the prenatal diagnosis of genetic abnormalities in fetal isolated mild ventriculomegaly.

Materials and methods: This retrospective study reviewed 101 fetuses with isolated mild ventriculomegaly who had undergone invasive prenatal diagnosis at our hospital. CMA was performed in all cases to detect chromosomal aneuploidy as well as copy number variations (CNVs) that are too small to be detected by conventional karyotyping. Real time quantitative PCR (qPCR) or multiplex ligation dependent probe amplification (MLPA) was used to confirm all fetal CNVs <400 Kb.

Results: Except for three cases of chromosomal aneuploidy, CMA revealed pathogenic copy number variations (CNVs) in 3.0% (3/101) of the fetuses; these cases demonstrated involvement in the chromosomal regions 15q11.2, 1q21.1 and Xq27.3q28. Furthermore, we detected three likely pathogenic (3.0%) and two variants of uncertain significance (2.0%) among 101 fetuses diagnosed as isolated mild ventriculomegaly on ultrasound examination.

Conclusion: Our study suggests that CNVs could aid in the risk assessment and genetic counseling in fetuses with isolated ventriculomegaly.

Introduction

Fetal ventriculomegaly is a relatively common symptom in prenatal ultrasound examinations. It is defined as mild when the unilateral or bilateral atrial diameter is between 10 and 15 mm and severe when the width is greater than 15 mm [1]. If no additional fetal structural anomalies are detected at the time of initial presentation on ultrasound examination, the ventriculomegaly is considered as an isolated abnormality. Compared with the poor prognosis associated with the presence of severe ventriculomegaly, most fetuses with isolated mild ventriculomegaly may have normal outcomes. The prevalence of neurodevelopmental delay was found to be 7.9–12% in fetuses with isolated mild ventriculomegaly, while only 2–3% in the general population [2]. Therefore, isolated mild ventriculomegaly is thought to be linked to an abnormal neurodevelopmental outcome.

Fetal ventriculomegaly is a dynamic phenotype. Previous study showed that the prenatal regression was observed in about 30% of fetuses with ventriculomegaly, persistence in 55% of cases and progression in 15% [3]. Furthermore, the ventriculomegaly was not truly “isolated” in some cases, which may be since the first sign of brain anomalies are recognizable only in the third trimester or even following delivery [4]. Fetal brain MRI and serial ultrasound scanning are suggested to follow up on fetal development and any progression of the ventriculomegaly, while pregnancy outcomes can still not be estimated exactly by depending solely on such imaging examinations. The diagnosis of isolated mild ventriculomegaly not only generates anxiety and uncertainty for pregnant women but also poses a counseling challenge for clinicians.

The widespread use of chromosomal microarray analysis (CMA) in patients with neurodevelopmental disorders has led to the discovery of new microdeletion/microduplication syndromes and the identification of candidate genes responsible for the clinical phenotypes. It was estimated that ~14.2% of the disease's presence in children with intellectual disabilities and various congenital defects was due to a prevalence of CNVs>400 Kb [5]. Previous studies have implied that prenatal CMA was identified in association with additional clinically significant abnormalities in approximately 6% of fetuses with ultrasonographic abnormalities and

* Corresponding author.
E-mail address: jie1967@126.com (J. Li).

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normal karyotypes [6], but limited data was available when only isolated mild ventriculomegaly was present.

Ruling out the microdeletion/microduplication syndrome may improve prenatal diagnosis and the prognostic evaluation of fetal ventriculomegaly. In this study, we analyzed the clinical data of 101 fetuses with isolated mild ventriculomegaly disgnosised on ultrasound examination and investigated whether CNVs contribute to the risk for developing isolated mild ventriculomegaly.

Materials and methods

Subjects

This retrospective study included 101 fetuses with isolated mild ventriculomegaly had undergone invasive prenatal diagnosis at our hospital from January, 2013 to February, 2017. The maternal age was 28.4 ± 4, and the gestational age at initial presentation of ventriculomegaly was 29 ± 3.5 weeks. Among the total number of cases, 6.9% (7/101) women had a past obstetrical history: five women experienced spontaneous abortion and two women had a pregnancy history of severe ventriculomegaly. All participants had provided written informed consent for the clinical prenatal diagnosis and further research.

Ultrasound scanning

The ventricular atrium width was measured at the level of the atria of the lateral ventricles which is filled by the echogenic choroid plexi, visible in an axial plane of the fetal brain from which also can be observed the frontal horns of the lateral ventricles and the cavum septi pellucidi. The calipers were positioned on the internal margin of the medial and lateral walls of the atria, at the level of the glomus of the choroid plexus, on an axis perpendicular to the long axis of the lateral ventricle. The anatomy scans were performed at the same time. When the unilateral or bilateral atrial diameter was 10–15 mm and no additional fetal structural anomalies were detected, isolated mild ventriculomegaly was considered. Fetal brain MRI and serial prenatal ultrasound examination were suggested to all pregnant women.

Chromosomal microarray analysis

Genomic DNA was extracted from cord blood using QIAamp® DNA Blood Mini Kit (Qiagen, Inc., Hilden, Germany) or from the amniotic fluid cells using BioChain Amniotic Fluid Genomic DNA Kit (BioChain, CA, USA). The CytoScanTM 750 K. Array (Affymetrix, Inc., Santa Clara, CA, USA) was used to detect microdeletions and microduplications according to the manufacturer's instructions. Gains and losses that affected a minimum of 50 markers in a 100 Kb length of chromosomal DNA were initially considered. Multiplex ligation dependent probe amplification (MLPA) was performed to confirm the CNVs at 22q11.2 (SALSA MLPA probemix P250-B2 Digeorge, MRC-Holland, The Netherlands), and real time quantitative PCR (qPCR) was used to confirm other fetal CNVs<400 Kb. The CNVs were classified as benign, pathogenic, likely benign, likely pathogenic or variants of uncertain significance (VOUS). Once the CNVs were detected in fetuses, we analyzed the parents' DNA using qPCR to determine whether the CNVs were inherited or de novo if parental material were available. The qPCR Primers were shown in Supplementary Table 1.

Results

Case characteristics

Prenatal ultrasound follow-up data was available in 64.4% (65/101) cases, in which 10.8% (7/65) progressed to severe ventriculomegaly and 52.3% (34/65) were alleviated. Additional brain abnormalities were detected in 10.9% (6/55) cases by prenatal brain MRI, including three cases of agenesis of the corpus callosum, one of cortical dysplasia, one of cerebellar vermis dysplasia, and one of increased basal ganglia. Among 101 fetuses with ventriculomegaly, nine (8.9%) cases were lost in the follow-up, 15 (14.9%) terminated the pregnancy, and 77 (76.2%) were delivered.

Chromosomal aneuploid in fetuses with isolated mild ventriculomegaly

The CMA identified chromosomal aneuploidy in 3.0% (3/101) of the cases, including one case of trisomy 21 and two cases of X-chromosome aneuploidy. For the three cases, only mild ventriculomegaly was detected on ultrasound examination, and no ultrasound follow-up data or prenatal fetal brain MRI results were available due to the termination of pregnancy (Table 1).

CNVs in fetuses with isolated mild ventriculomegaly

The CMA revealed 3.0% (3/101) pathogenic CNVs in fetuses diagnosed as isolated mild ventriculomegaly on ultrasound examination. Furthermore, three likely pathogenic CNVs and two VOUS were detected. The 177 Kb duplication at 22q11.2 was confirmed using MLPA, and the 316 Kb duplication at 17p13.3 was certified by qPCR. The inheritance status was not tested in Cases 42 and 43, because the parents' samples were unavailable. The details of the microarray nomenclature, clinical significance and the inheritance status were described in Table 2.

Case 43 was a male fetus with duplication at Xq27.3q28 involving MECP2 duplication syndrome. Duplications of Xq27.3q28 have little or no phenotypic significance in females due to the X-inactivation of the abnormal X-chromosome; nevertheless, males with this duplication are severely impaired [7]. Case 50 carried an inherited 1.86 Mb deletion involving the 1q21.1 Deletion syndrome. The phenotype associated with 1q21.1 Deletion syndrome is highly variable, ranging from asymptomatic to severe developmental delay and multiple congenital anomalies [8]. Case 87 revealed an inherited 507 Kb deletion associated with 15q11.2 Microdeletion syndrome. The deletion region contains four non-imprinted biallelically expressed genes (TUBGCP5, CFYIP1, NIPA1 and NIPA2) that are known to impact severity on clinical presentations and neurological impairments in the Prader-Willi/Angelman syndrome.

Table 1
Three chromosomal aneuploidies detected in fetuses with mild isolated ventriculomegaly.

Case	Fetal gender	Gestational age at initial presentation (in weeks)	US findings at the time of initial presentation	Microarray results	Interpretation	Outcome
63	Female	24	LA,10.8 mm; RA,10.4 mm	arr [hg19] (X)×3	Trisomy X	TOP
77	Female	26	LA,11.5 mm; RA,7.9 mm	arr [hg19] (X)×4	Tetraploid X	TOP
89	Male	32	LA,11.0 mm; RA,8.5 mm	arr [hg19] (21)×3	Trisomy 21	TOP

US, ultrasound; RA, right atrium; LA, left atrium; TOP, termination of pregnancy.

Table 2
Pathogenic CNVs, likely pathogenic CNVs and VOUS detected in fetuses with isolated mild ventriculomegaly.

Case	Microarray results	Size	Critical Genes/ syndromes involved	Prenatal MRI additional findings	Clinical significance	Inheritance status	Outcomes
13	arr [hg19]2q37.1q37.3 (234,689,089-238,879,302)×1	4.19 Mb	COL6A3	Cerebellar Vermis dysplasia	Likely pathogenic	Inherited	TOP
36	arr [hg19]17p13.3 (1,180,450-1,496,540)×3	316 Kb	YWHAE	None	Likely pathogenic	*De novo*	Livebirth; no abnormal phenotypes
42[a]	arr [hg19]22q11.21 (19,606,702-19,783,724)×3	177 Kb	TBX1	NA	Likely pathogenic	NA	TOP
43	arr [hg19]Xq27.3q28 (147,006,486-155,156,602)×2	8.15 Mb	MECP2 Duplication syndrome	NA	Pathogenic	NA	TOP
50	arr [hg19]1q21.1q21.2 (146,023,922-147,885,600)×1	1.86 Mb	1q21.1 Deletion syndrome	None	Pathogenic	Inherited	Livebirth; no abnormal phenotypes
87	arr [hg19]15q11.2 (22,770,421-23,277,436)×1	507 Kb	15q11.2 Deletion syndrome	Agenesis of the corpus callosum	Pathogenic	Inherited	TOP
92	arr [hg19]Xp22.31 (6,455,151-8,134,649)×2	1.68 Mb	STS	None	VOUS	Inherited	Livebirth; no abnormal phenotypes
98	arr [hg19]10q21.1 (54,300,230-54,804,477)×3	504 Kb	MBL2	None	VOUS	Inherited	Livebirth; no abnormal phenotypes

TOP, termination of pregnancy.
[a] A prenatal progression to severe ventriculomegaly was observed.

A heterozygous deletion at 15q11.2 may increase susceptibility to neuropsychiatric or neurodevelopmental problems [9,10].

Discussion

Before CMA technology developed, chromosomal karyotyping was the common genetic prenatal diagnosis method for fetuses with ventriculomegaly. The incidence of chromosomal anomalies among the fetuses with ventriculomegaly was 2.8–8.3%, depending on different study populations, screening protocols, and the diagnostic accuracy of the prenatal ultrasound scan [1,11]. It is generally believed that non-isolated and severe ventriculomegaly are more likely associated with chromosomal abnormalities than isolated mild ventriculomegaly, leading to lower rates of invasive procedure being performed on isolated mild cases [12]. The present study applied the CMA to fetuses with isolated mild ventriculomegaly to explore the relationship between mild ventriculomegaly and CNVs. To focus on the isolated cases, fetuses with additional structural anomalies were excluded such as heart defects, renal anomalies, or skeletal dysplasia. As a result, except for three cases of chromosomal aneuploidy, pathogenic CNVs were detected in 3.0% of the isolated mild cases diagnosed on prenatal ultransound. This supported the use of the CMA in prenatal genetic diagnosis for mild ventriculomegaly, and presented the underlying genetic etiology for isolated mild ventriculomegaly.

The interpretation of CNVs in prenatal cases is more challenging than in postnatal population because of the limitation in information on the clinical phenotype, especially for fetuses with neurodevelopmental abnormalities. Isolated mild ventriculomegaly has been considered a soft marker for chromosomal abnormalities rather than a fetal structural abnormality. Our study showed that the ventriculiomegaly in 52.3% of mild cases was alleviated before birth. Therefore, it would be more prudent to estimate the pathogenicity of the CNVs for isolated mild cases.

Among three pathogenic CNVs detected, the only CNV with an easily predictable abnormal phenotype is the Xq27-28 dup that contains MECP2. The 15q11.2 microdeletion (Case 87) and 1q21.1 microdeletion (Case 50) were inherited from parents without obvious clinical features. The 15q11.2 Microdeletion syndrome has a reported de novo frequency of between 5% and 22%, with 51% of cases having inherited the microdeletion from an apparently unaffected parent [9]. The penetrance of the 15q11.2 microdeletion was estimated at 10.4% [13]. For Case 87, the agenesis of the corpus callosum detected on MRI followed-up implied the higher possibility of abnormal phenotype, but no other phenotypes could be gained because the parent chose the termination of pregnancy and refused an autopsy. The 1q21.1 microdeletion is inherited in an autosomal dominant manner, with 50%–82% inherited from a parent with a normal phenotype, or an abnormal phenotype that is similar to but usually less severe than that of his/her child [14]. Little information was available regarding penetrance of the 1q21.1 microdeletion; however, the lower de novo frequency suggested it has a reduced penetrance. No abnormal clinical phenotypes were shown in Case 50 until the baby was six months old, while the further long-term follow-up was needed. Although pathogenic CNVs were carried in Cases 87 and 50, it cannot be completely predicted what phenotypes would be shown considering the incomplete penetrance along with variable expressivity.

Case 13 had a 4.19 Mb interstitial deletion at 2q37.1q37.3. Although the deletion of COL6A3 involved in the region is associated with Bethlem myopathy 1, a rare autosomal dominant proximal myopathy with an early childhood onset [15], it could not be certified as a pathogenic CNV because of the clinically normal father carrying the same deletion. The YWHAE gene involved in Case 36 and TBX1 gene involved in Case 42 were reported as critical genes of 17p13.3 Duplication syndrome and 22q11.2 Microduplication syndrome respectively. However, the common 17p13.3 Duplication syndrome and 22q11.2 Microduplication syndrome are much larger in size than duplications in Cases 36 and 42 [16–18]. The deletions/duplications of Cases 13, 36 and 42 were defined as likely pathogenic CNVs, because insufficient evidence was available to unequivocally determine its clinical significance.

Mechanisms by which the CNVs may influence disease risk were complex and diversified. A gene associated with a clinical phenotype due to haploinsufficiency may not possess a phenotype associated with a copy number gain. It is known the deletion at Xp22.31 is associated with X-linked ichthyosis, whereas it is challenging to categorize the duplication at this region [19]. The duplication at Xp22.31 has been reported as being a benign variant with a general population frequency of 0.15%, while Li et al. [20] suggested that Xp22.31 duplication might contribute towards a phenotype which includes developmental delay, intellectual disability and autism. For Case 92, only isolated mild ventriculomegaly was detected on prenatal ultrasound examination, and the infant showed normal development until eight months old, nevertheless, it is uncertain whether the Xp22.31 duplication would lead to long-term neurodevelopmental abnormalities.

Our study has some limitations. Because of its retrospective nature, the inheritance status of CNVs, ultrasound follow-up data or

pregnancy outcomes were unavailable in some cases. In addition, more subtle disabilities may become apparent at school age, thus it is difficult to link CNVs with the current phenotype. An enhanced interpretation of the results is expected with increased knowledge of the human genome and improved databases on the relationship between clinical phenotypes and CNVs.

Conclusion

Among the 101 fetuses with isolated mild ventriculomegaly, CMA detected three exhibited chromosomal aneuploidy and three had pathogenic CNVs, implying that CNVs could aid in the risk assessment and genetic counseling in fetuses with isolated ventriculomegaly. As a complementary method for imaging examination and chromosomal karyotyping, the CMA was demonstrated as providing additional value in the prognostic evaluation of fetuses with isolated ventriculomegaly, while it should be prudent to estimate the pathogenicity of the CNVs considering the limited clinical phenotypes on prenatal cases.

Conflicts of interest

There is no conflict of interest in this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tjog.2019.01.015.

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