Clin. Lab. 2017;63:XXX-XXX

CASE REPORT

A Novel Case of 15q24 Microdeletion Syndrome Detected by MLPA in a Chinese Family

Xiao-Ying Dai [1, 2, *], Lu Zhou [1, *], Yang Liu [1], Jian-Sheng Xie [1]

** These authors contributed equally to this study*
[1] Medical Genetics Laboratory, Shenzhen Maternal and Child Health Hospital, Guangdong, China
[2] Prenatal Diagnosis Center, The University of Hong Kong-Shenzhen Hospital, Shenzhen, Guangdong, China

SUMMARY

Background: **Chromosome 15q24 microdeletion syndrome is a rare disease. To date, only 40 cases have been reported. Here, we also confirmed a 15q24 microdeletion syndrome in a chorionic villus of miscarriage.**
Methods: **The microdeletion was screened by multiplex ligation-dependent probe amplification (MLPA) and then identified by chromosomal microarray analysis (CMA).**
Results: **A 15q24 microdeletion syndrome was screened by MLPA in the chorionic villus of miscarriage in a Chinese family and was confirmed to be a *de novo* 3.143 Mb 15q24.1q24.2 deletion (chr15:72930195-76073450) by chromosomal microarray analysis (CMA).**
Conclusions: **We first reported the 15q24 microdeletion syndrome screened by MLPA in Chinese population, and we also considered that the technique of MLPA with a suitable kit and probe could screen such a rare microdeletion quickly, economically, and efficiently.**
(Clin. Lab. 2017;63:xx-xx. DOI: 10.7754/Clin.Lab.2017.170220)

Correspondence:
Dr. Jian-Sheng Xie
Medical Genetics Laboratory
Shenzhen Maternal and
Child Health Hospital
518028 Shenzhen, Guangdong
China
Phone: +86 755 86913333 ext.7003
Fax: +86 755 86913333 ext.7003
Email: jsxieszmch@aliyun.com

Case Report accepted March 5, 2017

KEY WORDS

15q24 microdeletion syndrome, multiple ligation probe amplification (MLPA), chromosomal microarray analysis (CMA), low-copy repeats (LCRs)

INTRODUCTION

Chromosome 15q24 microdeletion syndrome is a rare disease that was first analyzed by Sharp in 2007. This disease is a recent emerging syndrome mainly identified due to widespread use of array-CGH and the overall incidence in the general population may approach approximately 1 in 42,000 [1]. So far, 40 cases have been reported, all of which were *de novo* and males [2,3]. The majority of 15q24 deletions have breakpoints that localize in one of five low-copy repeat (LCRs) clusters labeled LCR15q24A, -B, -C, -D, and -E, and the smallest region of overlap (SRO) spans a 1.2 Mb region between LCR15q24B to LCR15q24C [4]. The deletion identified in this study was also in the SRO region.
We first reported the 15q24 microdeletion syndrome detected by MLPA in a chorionic villus of miscarriage in

Chinese population, and then confirmed it a *de novo* because of no abnormalities were detected in the parents by conventional karyotype analysis, MLPA, and CMA.

MATERIALS AND METHODS

MLPA

Genomic DNA was extracted from peripheral blood according to standard procedures (QIAamp DNA Blood Mini Kit Handbook, QIAGEN), and the microdeletion was detected by MLPA according to instructions of SALSA MLPA probe mixes of P245-B1, P036-E2, and P070-B3 (MRC-Holland) for all samples. All initial data of MLPA were analyzed by excel-based Coffalyser, the allele copy numbers were determined by cutoff values (lower than 0.7).

CMA

Genomic DNA was extracted from blood lymphocytes using the QIAamp DNA Blood Midi kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. DNA concentration was determined with a NanoDrop ND-2000 spectrophotometer and software (NanoDrop Technologies, Berlin, Germany). Detection of gene copy number was performed by Affymetrix Cyto750K Array chip following standard and manufacturer's recommendations. All data were performed using Affymetrix Software and analyzed with the references from the database of Genomic Variant (DGV), database of DECIPHER, ISCA, and OMIM to confirm whether the CNVs found in the sample were pathogenic. All genomic locations correspond to NCBI build 36 (hg19).

RESULTS

The pedigree analysis of this family with 15q24 microdeletion syndrome was performed (Figure 1). It involved three miscarriage embryos which were all males. The subject III-4 which was aborted after 12 weeks was the proband of 15q24 microdeletion, and was identified by MLPA and CMA. Subjects of II-3 and III-1 were aborted embryos of 20 weeks and 18 weeks old, respectively, which might have been caused by some malformations discovered by ultrasonography but without laboratory examinations. The maternal individuals were all normal.

The MLPA results of the proband indicated that the miscarriage subject was male, and the chart had only two signal peaks decreased to 0.53 (detecting gene SEMA7A) and 0.55 (gene CYP1A1), which revealed a 15q24 microdeletion in the miscarriage proband (see Figure 2 - 3), while the blood samples of his parents were both normal (data not shown).

The CMA analysis of the miscarriage proband revealed a deletion at chromosome region 15q24.1 - 15q24.2, located on low-copy repeat (LCRs) clusters from LCR 15q24B to LCR15q24C. This deletion also included the smallest region of overlap (SRO) like most reported cases. The minimum size of the deletion was estimated as 3.143 Mb from the probe at 15q24.1 (chr15: 72,930,195 bp) to the probe at 15q24.2 (chr15: 76,073,450 bp) (Figure 4). This detection involved 60 genes in total, 37 of which were OMIM genes: gene STRA6, CYP11A1, SEMA7A, CPLX3, ARID3B, SIN3A, CSK, and so on (details not shown here). The results of chromosomal G-banding, MLPA, and CMA analysis of the parents were all normal (data not shown). Thus, the 15q24 microdeletion observed in this miscarriage appeared to be *de novo*.

DISCUSSION

As we all know, the 15q24 microdeletion syndrome is inherited in an autosomal dominant manner, which is characterized by growth retardation, intellectual disability, distinct facial features, digital abnormalities, loose connective tissue, congenital malformations, genital malformations in males, and other diverse phenotypes. Although this syndrome was reported occasionally in survival individuals, it has never previously been published in miscarriage products, and so this case was the first one, consequently, the syndrome could lead to miscarriage or diverse dysmorphia. However, the recurrence risk for future pregnancy of this couple is difficult to estimate (probably < 1%) because they do not have this deletion [3]. If there were some possibilities of the parental germline mosaicism or a balanced chromosomal rearrangement involving the 15q24 region but not detected by the CMA, the risk will be greater than that of general population. The other two miscarriages in this pedigree were suspected to be 15q24 microdeletion because of their abnormal ultrasonic B and the male gender. But meanwhile, other chromosome aberrations in the two miscarriages could not be excluded due to lack of laboratory tests.

To our knowledge, there are several candidate genes within the SRO, including CYP11A1, SEMA7A, CPLX3, ARID3B, STRA6, SIN3A, and CSK, that may predispose to many of the clinical features observed in individuals with 15q24 deletion syndrome [4,5]. In this study, the molecular basis of the miscarriage was the absence of 60 genes (OMIM genes 37), involving such candidate genes in the SRO regions that play an important role in different tissue organs such as brain, genital, cardiovascular, embryonic development, neoplasia, and so on. The phenotype, clinical features, and outcomes of 15q24 deletion will depend on the size, location, and genes involved in the chromosome abnormality [6].

Although the kit of SALSA probemix P245-B1 (MRC-Holland) could only detect the absence of gene CYP1A1 and SEMA7A, the majority of reported cases of 15q24 deletion have included such two genes in their deletion segments. Therefore, the MLPA of P245-B1 kit could be used as the first screening method for suspect-

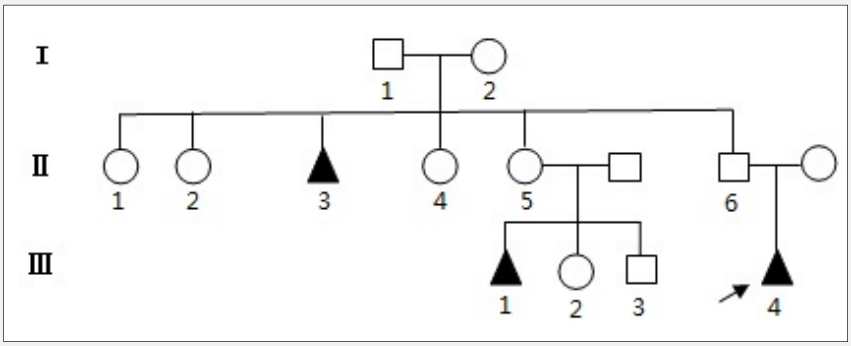


Figure 1. The pedigree chart of the family with 15q24 microdeletion syndrome.

Black triangle with an arrow: proband, black triangles: aborted embryos suspected with 15q24 deletion, open circles: normal females, open squares: normal males.

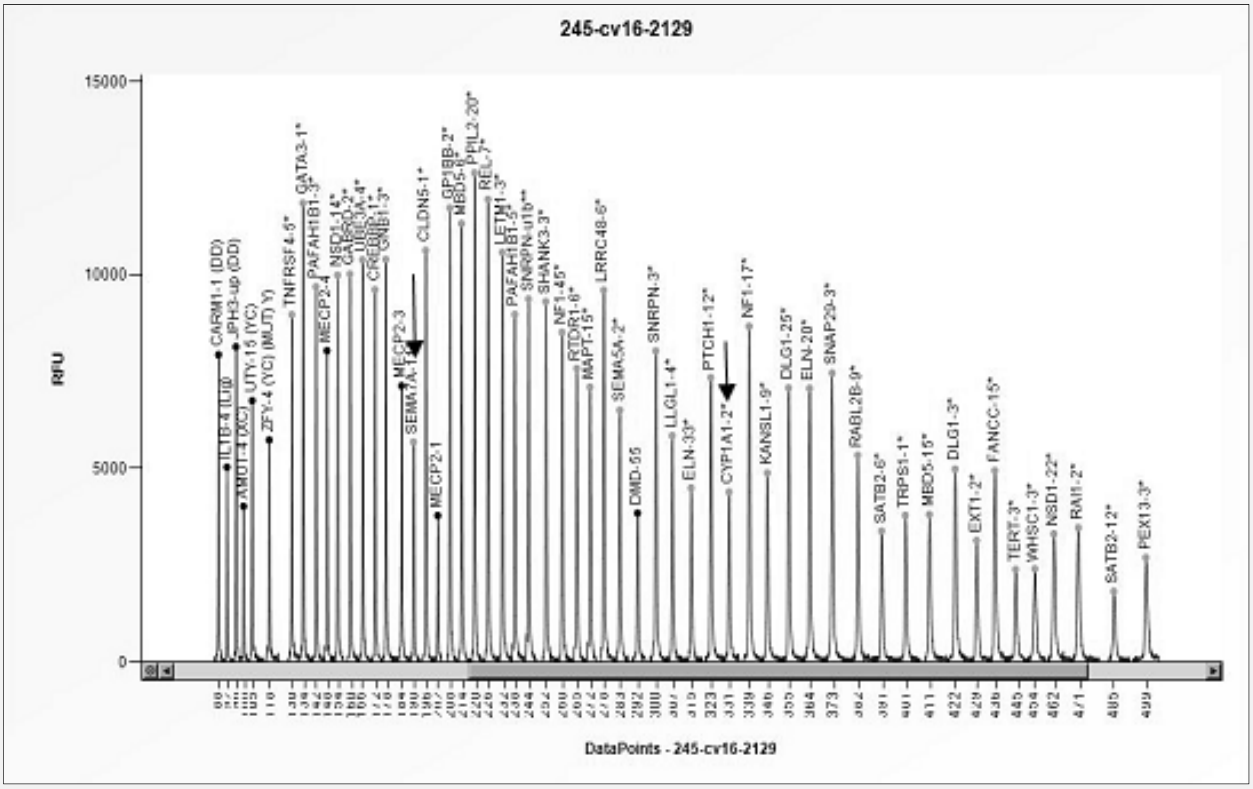
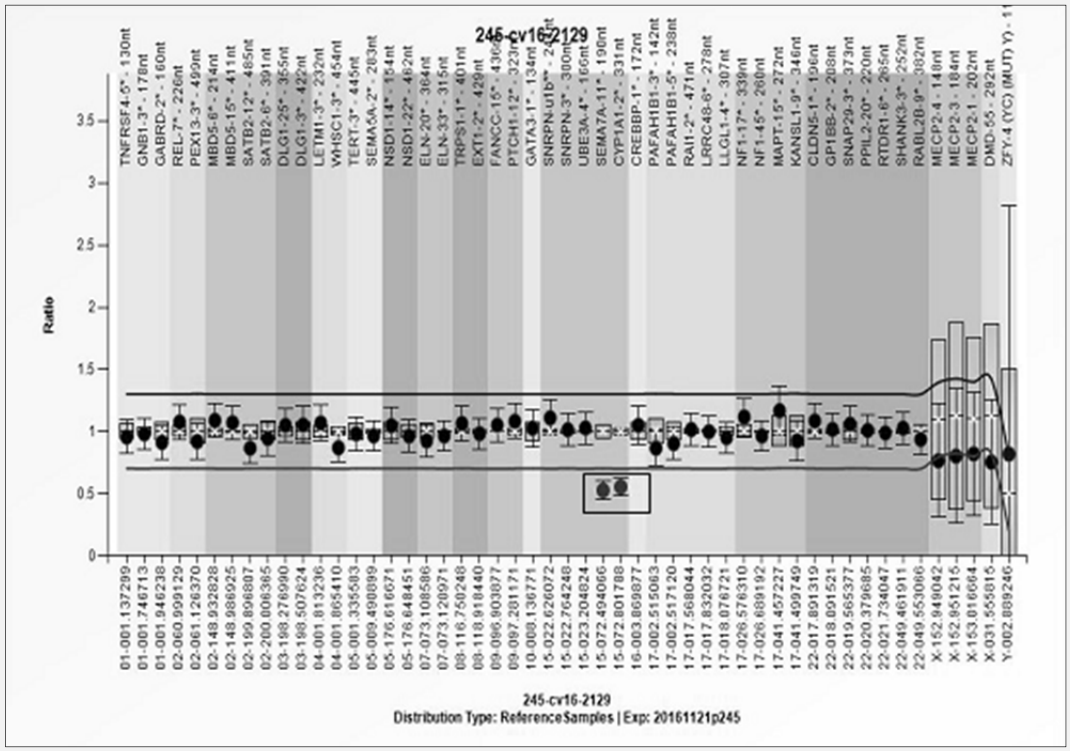


Figure 2. The MLPA raw data of the miscarriage proband.

The arrows demonstrated two signal peaks decreased to half, while others were normal.



D [nt]	Gene-Exon	Chr.band	hg18 loc.	Height	Area	Ratio	Stdev	[REF]	[Sam]	Width	d[nt]
436	FANCC-15*	09q22.32	09-096.903877	4950	60476	*1.05*	0.07	=	=	40	0.2
323	PTCH1-12*	09q22.32	09-097.281171	7350	65406	*1.08*	0.07	=	=	59	-1.0
134	GATA3-1*	10p14	10-008.136771	11864	57662	*1.03*	0.08	=	=	38	-0.1
244	SNRPN-u1b**	15q11.2	15-022.626072	9384	64098	*1.11*	0.07	=	=	44	0.2
300	SNRPN-3*	15q11.2	15-022.764248	8042	65436	*1.01*	0.06	=	=	54	-0.7
166	UBE3A-4*	15q11.2	15-023.204824	10394	54297	*1.03*	0.07	=	=	45	0.1
190	SEMA7A-11*	15q24.1	15-072.494066	5688	32344	*0.53*	0.04	<<	<<	32	0.0
331	CYP1A1-2*	15q24.1	15-072.801788	4392	38758	*0.55*	0.04	<<	<<	41	-0.9
172	CREBBP-1*	16p13.3	16-003.869877	9624	49270	*1.05*	0.08	=	=	34	0.2
142	PAFAH1B1-3*	17p13.3	17-002.515063	9700	47165	*0.86*	0.07	=	=	29	0.0
238	PAFAH1B1-5*	17p13.3	17-002.517120	8984	59209	*0.9*	0.06	=	=	31	0.1
471	RAI1-2*	17p11.2	17-017.568044	3470	49176	*1.02*	0.07	=	=	59	-0.8
278	LRRC48-6*	17p11.2	17-017.832032	9812	69720	*1*	0.06	=	=	38	-0.1

Figure 3. The two diagrams analyzed by software Coffalyser showing the gene SEMA7A and CYP1A1 deletion (rectangle marked) and the Ratio of the signals 0.53 and 0.55, respectively, (lower than cutoff 0.7).

ed microdeletion patients or miscarriage. This method will save patients much more time and money than CMA or aCGH and could be widely used in screening. But for parents of probands and recurrent spontaneous abortions with normal MLPA results, it is strongly recommended that CMA or aCGH testing should be applied in order to confirm that they carry neither the deletion of any breakpoint nor other imbalanced rearrangement in 15q24 region [7,8]. In spite of that, there are still some missed detections of germline mosaicism.

CONCLUSION

Individuals with 15q24 deletion syndrome have a 50% chance of passing the deletion on to their offspring. Whether the descendants are survivals (with diverse phenotypes, clinical features, and prognosis) or miscarriage will depend on the size and location of the deletion, and genes involved in the chromosome abnormality. The technique of MLPA, compared to CMA, FISH or aCGH, can be used as the first choice to screen this microdeletion, because it is much faster and more eco-

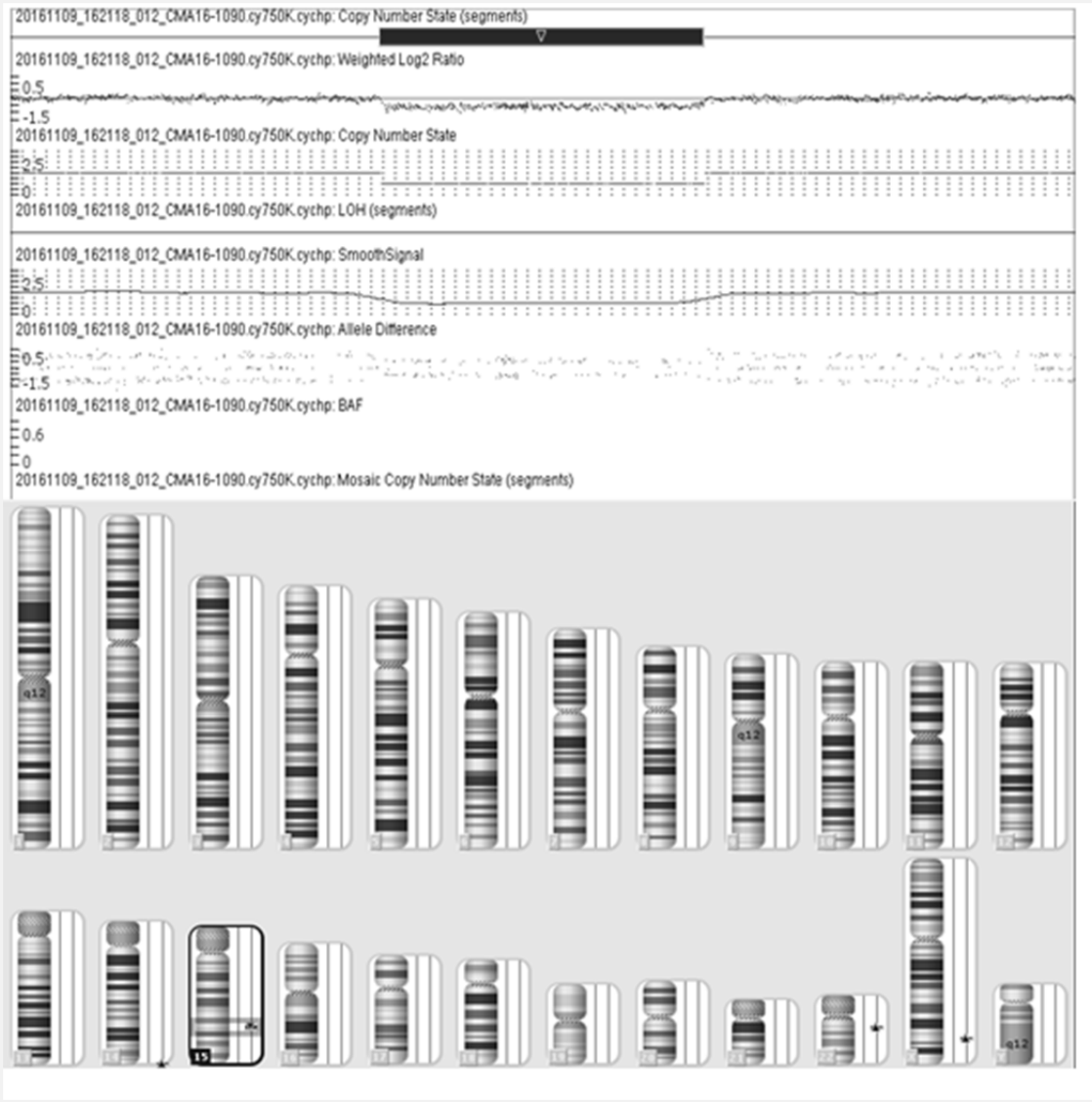


Figure 4. The array data (using Affymetrix cyto750k chip) analysed by Affymetrix Software showing a 3.143 Mb deletion at 15q24.1q24.2.

All genomic locations correspond to NCBI build 36 (hg19). The wide bar above and rectangular box below represented the deleted region in this case (72.930 - 76.073 Mb) of the miscarriage sample. The others' CNVs were benign or as yet unknown.

nomical. In addition, it includes the common genes in SRO region. So, this method can be used in initial screenings of all miscarriage samples. When detecting highly suspected cases with the normal MLPA results, we can do the analysis of array-CGH or FISH to exclude other imbalanced rearrangement or the region deletions of LCR15q24A, -D or -E.

Acknowledgement:
This study was supported by the Natural Science Foundation of China (Grant Number: 31471204) and Shenzhen Municipal Committee of Science and Technology Innovation (Grant Number: JCYJ20150402090413006).

Declaration of Interest:
The authors report no conflict of interests.

References:

1. Magoulas PL1, El-Hattab AW. Chromosome 15q24 microdeletion syndrome. Orphanet J Rare Dis 2012;7:2 (PMID: 22216833).

2. Palazón-Bru A, Ramírez-Prado D, Cortés E, Aguilar-Segura MS, Gil-Guillén V. An inferential study of the phenotype for the chromosome 15q24 microdeletion syndrome: a bootstrap analysis. PeerJ 2016; 4:e1641 (PMID: 26925314).

3. Law HY, Brett M, Tan EC, Yong MH, Lai A. AB024. Chromosome microarray analysis (CMA) for the diagnosis of children with developmental delay and multiple congenital anomalies in Singapore. Ann Transl Med 2015;3 (Suppl 2):AB024. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4563548.

4. Mefford H, Shur N, Rosenfeld J. 15q24 Microdeletion Syndrome. In: Pagon RA, Adam MP, Ardinger HH, et al, editors. Gene Reviews® [Internet]. Seattle (WA): University of Washington, Seattle, 1993-2016, 2012 Feb 23 (PMID: 22359776).

5. Watson CT, Marques-Bonet T, Sharp AJ, Mefford HC. The Genetics of Microdeletion and Microduplication Syndromes: An Update. Annu Rev Genomics Hum Genet 2014;15:215-44 (PMID: 24773319).

6. El-Hattab AW, Zhang F, Maxim R, et al. Deletion and duplication of 15q24: molecular mechanisms and potential modification by additional copy number variants. Genet Med 2010;12:573-86 (PMID: 20860070).

7. Klopocki E, Graul-Neumann LM, Grieben U, et al. A further case of the recurrent 15q24 microdeletion syndrome, detected by array CGH. Eur J Pediatr 2008;167:903-8 (PMID: 17932688).

8. Narumi Y, Shiohara M, Wakui K, et al. Myelodysplastic syndrome in a child with 15q24 deletion syndrome. Am J Med Genet A 2012;158A(2):412-6 (PMID: 22140075).