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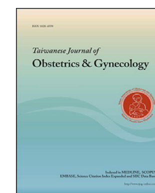

Short Communication

Detection of recurrent 4p16.3 microdeletion with 2p25.3 microduplication by multiplex ligation-dependent probe amplification and array comparative genomic hybridization in a fetus from a family with Wolf–Hirschhorn syndrome

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ABSTRACT

Objective: We present prenatal diagnosis, genetic counseling, and molecular cytogenetic features of familial recurrence of Wolf–Hirschhorn syndrome (WHS).
Materials and methods: A 31-year-old woman was referred to a hospital at 24 weeks of gestation because of abnormal ultrasound findings in the fetus. Her first child was a boy who had growth retardation, mental defect, and a distinctive facial appearance. Based on the conventional cytogenetic analysis, the combined use of multiplex ligation-dependent probe amplification (MLPA) and array comparative genomic hybridization (aCGH) facilitated the prenatal diagnosis and genetic counseling in the fetus. Results of the standard G-banging karyotype analysis of the fetus, the parents, and the boy were normal.
Results: The MLPA analysis revealed the same 4p microdeletion accompanied by 2p microduplication in the fetus and the boy. The aCGH analysis revealed a 3.57-Mb 4p16.3 microdeletion or arr [hg19] 4p16.3 (71,552–3,636,893) x1 in the fetus and a 3.29-Mb 4p16.3 microdeletion or arr [hg19] 4p16.3 (71,148–3,360,737) x1 in the boy. The 3.57-Mb 4p16.3 microdeletion encompassed 39 OMIM genes. The 3.29-Mb 4p16.3 microdeletion encompassed 36 OMIM genes. They both included *LETM1* and *WHSC1*. The 2p25.3 microduplication was smaller than 666 kb and encompassed only one OMIM gene, *ACP1*.
Conclusion: The combined use of MLPA and aCGH is an effective way to diagnose recurrent WHS. Although WHS is typically caused by a *de novo* deletion, prenatal diagnosis and genetic counseling are necessary in the next pregnancy in families that have suffered such cases.
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Introduction

Wolf–Hirschhorn syndrome (WHS; OMIM 194190) results from the deletion of contiguous genes of chromosome 4p [1,2]. Novel techniques, such as array comparative genomic hybridization (aCGH) and multiplex ligation-dependent probe amplification (MLPA), facilitate the evaluation of WHS with greater accuracy compared with fluorescence *in situ* hybridization [3–5], although fluorescence *in situ* hybridization is a detection tool used to study an individual with a strong clinical suspicion of chromosomal abnormality and a normal conventional cytogenetic study. Owing to the low recurrence rate of WHS, little is known about the risk assessment of recurrent cases. Here, we report the characteristics of one fetus and one patient with familial recurrence of WHS from China that were determined by using the combination detection of MLPA and aCGH. The purpose of this research is to report our experience in prenatal diagnosis and genetic counseling for recurrent WHS.

Materials and methods

Clinical description

A 31-year-old gravida 2, para 1 woman was referred to our hospital for genetic analysis at 24 weeks of gestation because of abnormal ultrasound findings. Her husband was 34 years old. They are nonconsanguineous couple. Their first child is a boy, with an age

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of 7 years and 5 months who has growth delay, moderate mental retardation, and a characteristic facial appearance. The boy's birth weight was 2000 g (<3rd percentile), and his birth length and head circumference were not noted. Psychometric assessment at 4 years of age using the Gesell Developmental Schedules showed that his adaptive ability developmental quotient (DQ) was 48.4, and his gross and fine motor ability DQs were 52.7 and 46.9, respectively. His language DQ was 49.2, and his personal–social DQ was 41.7. He had an obvious Greek warrior helmet facial appearance, including a triangular face, high forehead, prominent glabella, hypertelorism, high-arched eyebrows, protruding eyes, a short philtrum, a distinct mouth with downturned corners, micrognathia, and lightly dysplastic and protruding ears (Figure 1). At the age of 7, all of his growth parameters were below the third percentile. His language development was delayed, still limited to short sentences. Seizures did not occur during the course of the disease.

No complications were noted during this pregnancy until a routine ultrasound examination at approximately 24 weeks of gestation showed the presence of oligohydramnios, symmetrical intrauterine growth restriction, a single umbilical artery, and uncertain micrognathia. Therefore, the woman underwent umbilical cord blood test at 24 weeks' gestation. The pregnancy was terminated as requested by the parents at 27 weeks' gestation, after they had sought genetic counseling about the long-term complications and poor prognosis of this disease.

Informed consent was obtained from the parents of the fetus. The procedures followed in this investigation were approved by the Human Research Ethics Committee of the First Hospital of Peking University.

Conventional cytogenetic analysis

Routine G-banding technique at the 400 band of resolution was performed. The umbilical cord blood of the fetus and the peripheral blood of the boy and their parents were collected and cultured according to the standard cytogenetic protocol.

MLPA

Genomic DNA was extracted using QIAamp DNA mini kit (Qiagen, Valencia, CA, USA) from the core family members, including the umbilical cord blood of the fetus, as well as the peripheral blood of the boy and their parents. MLPA analysis was performed to screen for subtelomeric rearrangements using SALSA P070 and P036, microdeletion syndromes using SALSA P245, and then further investigated 2p using a SALSA P208-C1 probemix (MRC-Holland. Amsterdam, the Netherlands). All probes in P070 differed from the P036 probes. The P070 and P036 both contained one probe for 4p subtelomeric regions. The P245 contained two special probes for WHS. The P208-C1 contained 11 probes in the terminal 3.7 Mb of 2p. The MLPA analysis was performed according to the manufacturer's instructions [6]. The amplification products were identified and quantified by capillary electrophoresis using a genetic analyzer (ABI 3130XL, USA). The fluorescence signal intensities of the polymerase chain reaction products were determined with Gene Marker l.6 software.

aCGH

An Agilent Sure Print G3 Human CGH Microarray 8 × 60K Kit (Agilent Technologies, Santa Clara, CA, USA) was used for genetic analysis of the fetus and the boy according to the manufacturer's instructions, and this microarray included 55,077 probes with a median probe space of 41 kb for intergenic genomic sequences. DNA hybridization was performed according to the standard procedures after the labeling of 500 ng of the sample DNA with cyanine-5 and the labeling of control DNA (Promega, Madison, USA) with cyanine-3. The signals were captured according to the instructions of the Agilent Sure Print G3 CGH Microarray Kit. Microarray data were analyzed using Feature Extraction software and Workbench genomics software (Agilent Technologies). Probe alignments were performed using NCBI 37 (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov/), UCSC (University of California, Santa Cruz, CA, USA; http://genome.ucsc.edu/) hg19 build, and DECEPHER v8.8 (https://decipher.sanger.ac.uk/).

Results

The standard G-banding karyotypes of this family were normal. The MLPA results of the parents were normal (Figure 2). The results of MLPA indicated a same 4p deletion accompanied by 2p

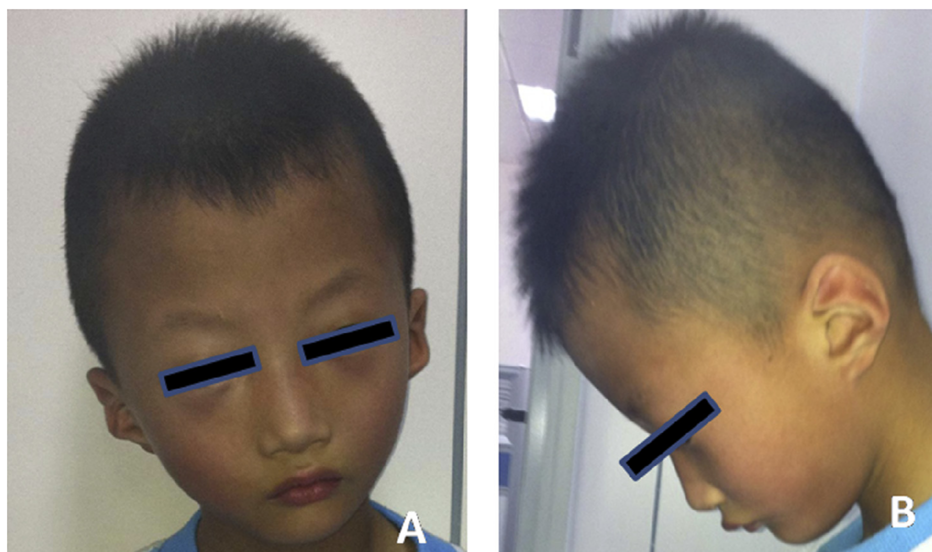

Figure 1. Features of facial dysmorphism of the boy at 7 years and 5 months of age.

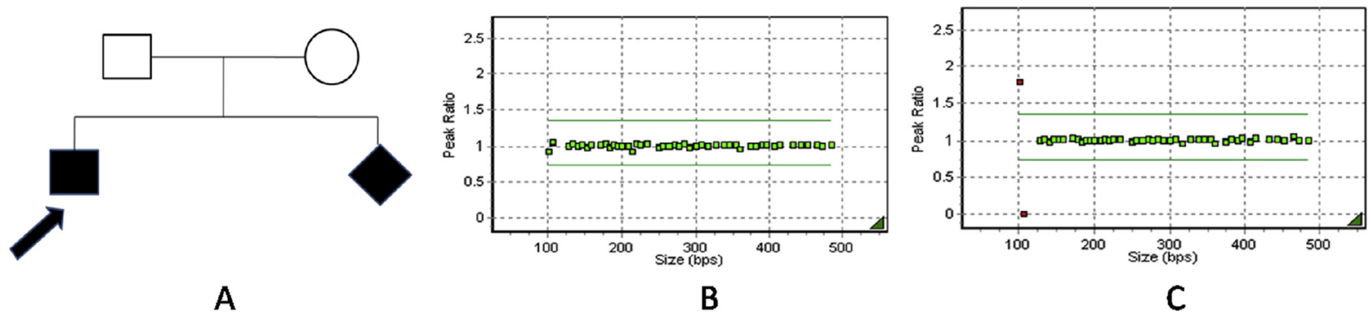

Figure 2. Pedigree diagram of the recurrent WHS family and MLPA results for the parents. (A) Pedigree of a Chinese family with WHS. Squares = males; circles = females; diamond = fetus; filled squares, circles and diamonds = affected members; arrow = proband. (B, C) Normal MLPA P070 results for the father and mother, respectively. MLPA = multiplex ligation-dependent probe amplification; WHS = Wolf–Hirschhorn syndrome.

Figure 3. Molecular diagnostic findings of the fetus. (A) The aCGH results showed a 3.57-Mb 4p16.3 microdeletion or arr [hg19] 4p16.3 (71,552–3,636,893) x1 in the fetus and (B) zoom-in view. (C–E). The MLPA P070, P245, and P208-C1 results for the fetus. The MLPA results showed 4p deletion accompanied by 2p duplication in the fetus. aCGH = array comparative genomic hybridization; MLPA = multiplex ligation-dependent probe amplification.

duplication in both the fetus (Figure 3) and the boy (Figure 4). The 2p25.3 duplication included specific probes 14154-L15754 and 14157-L15757. The duplicated region encompassed three genes, including *ACP1* (OMIM 171500). The size of the duplicated region was determined to be smaller than 666 kb based on the appearance of a signal at 255 kb and its disappearance at 666 kb from the distal 2p telomere. aCGH analysis revealed a 3.57-Mb 4p16.3 microdeletion or arr [hg 19] 4p16.3 (71,552–3,636,893) x1 in the fetus (Figure 3) and a 3.29-Mb 4p16.3 microdeletion or arr [hg19] 4p16.3 (71,148–3,360,737) x1 in the boy (Figure 4). The 3.57-Mb 4p16.3 microdeletion encompassed 39 OMIM genes. The 3.29-Mb 4p16.3 microdeletion encompassed 36 OMIM genes. They both included *LETM1* (OMIM 604407) and *WHSC1* (OMIM 602952). The results of the molecular cytogenetic investigations of their parents were normal.

Discussion

The recurrence rates of WHS offspring are typically low and dependent on the different sizes and categories of 4p deletion

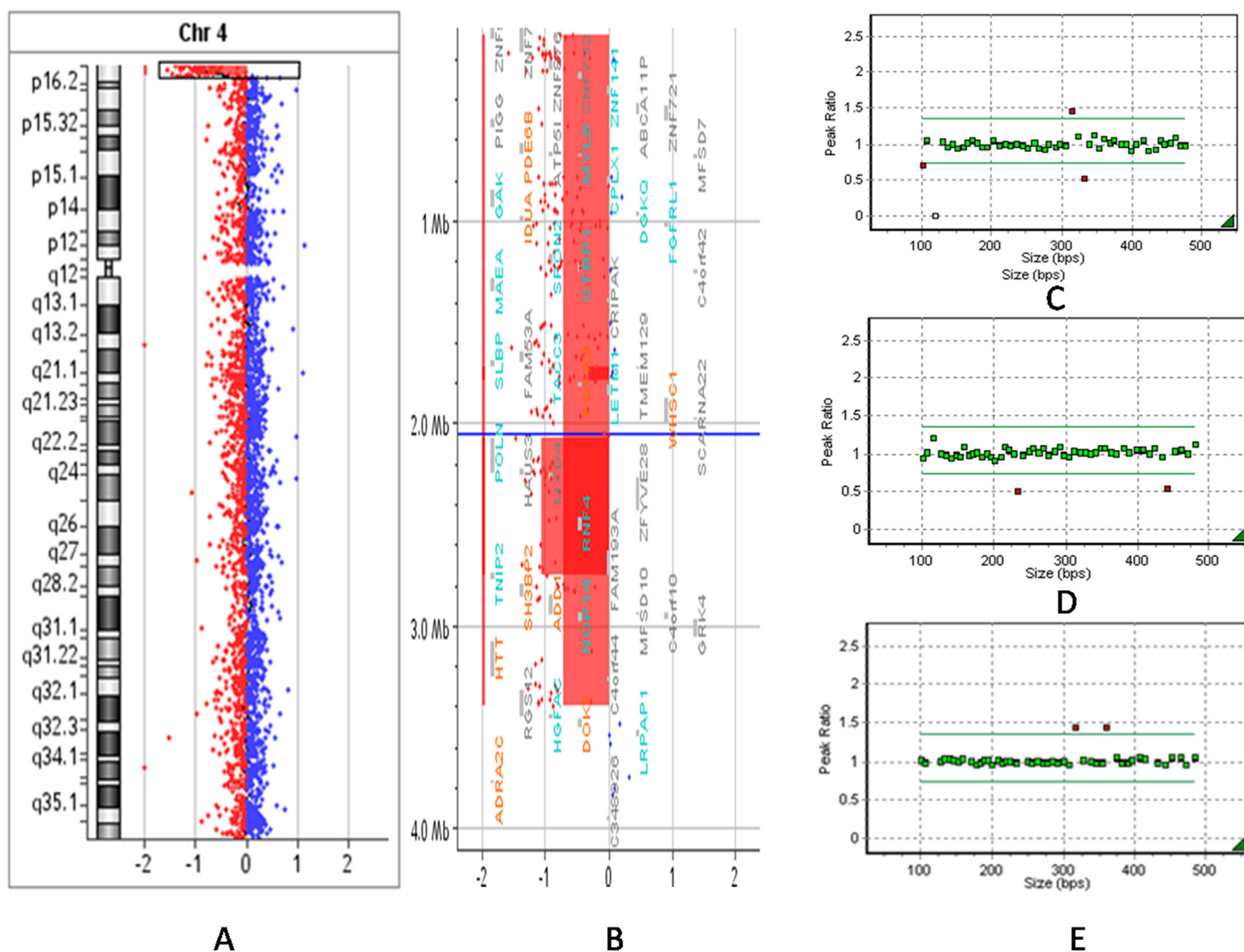

Figure 4. Molecular diagnostic findings of the boy. (A) The aCGH results showed a 3.29-Mb 4p16.3 microdeletion or arr [hg19] 4p16.3 (71,148–3,360,737) x1 in the boy and (B) zoom-in view. (C–E) The MLPA P070, P245, and P208-C1 results for the boy. The MLPA results showed 4p deletion accompanied by 2p duplication in the boy. aCGH = array comparative genomic hybridization; MLPA = multiplex ligation-dependent probe amplification.

[7]. Identification of the recurrent 4p deletion (4p16.3 → pter) and 2p duplication (2p25.3 → pter) in one family was a rarer finding. It can be concluded that the parents in this family had cryptic rearrangements, resulting in unbalanced gene dosages in their offspring that increased the recurrence rate of deletion syndromes. However, we failed to find other same reported cases. The absent expression of *WHSC1* has been associated with the typical craniofacial features of WHS and obvious growth delay [8]. *LETM1* has been reported to regulate ATP levels by acting on a protein involved in Ca^{2+}/H^{+} exchange in the mitochondria, affecting seizure activity in the brain [9]. The absence of seizures in the boy is probably because of his particular genetic aberration. Deletions of 4p are relatively common. However, duplication of the short arm of chromosome 2 is rare, and it seems can increase the severity of the short stature [10]. The features of partial duplication of the short arm of chromosome 2 were described in 1976 as “2p partial trisomy syndrome” [11] for the first time. This syndrome has several phenotypic features that overlap with those of WHS, including psychomotor delay and dysmorphic facial features. In our study, the size of 2p duplication was smaller than 666 kb; however, it was not detected by aCGH. The results of genetic investigation of the parents were normal. The main reason of the failure for aCGH to identify the duplication is that there are fewer genes on chromosome 2p compared with the other chromosomes and thus few probes for the 2p terminus. Only one OMIM gene was discovered in the duplicated region, that is, *ACP1*. Thus, we can conclude that the duplication of *ACP1*, the expression of which is negatively correlated with growth hormone levels [12], may also have caused intrauterine growth restriction and short stature observed in the fetus and the boy.

Prenatal diagnosis and genetic counseling are indispensible for preventing the recurrence of WHS. WHS is usually discovered by ultrasound with abnormal results and further confirmed by detection of 4p deletion through chromosome microarray analysis (CMA) [2,13]. The focus of the genetic counseling for recurrence of WHS should be noted as follows. (1) Fetuses with abnormal prenatal ultrasound and normal karyotype require further careful prenatal genetic counseling. (2) Once the karyotype is deemed normal, prenatal CMA should be considered prior to genetic counseling if the parents agree with the interventional tests. (3) The results of CMA may require other high-resolution genetic methods, such as MLPA, for further comprehensive analysis. (4) Parents should be encouraged to supply their samples for genetic analysis, which is a helpful way to diagnose whether the chromosomal variation of their fetus is harmful.

Conclusion

The combined use of aCGH and MLPA has increased the detection rate of submicroscopic chromosomal aberrations. Although WHS is typically caused by a *de novo* deletion, it is necessary to carry out prenatal diagnosis and genetic counseling in subsequent pregnancies in families that have suffered such cases.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Acknowledgments

We thank the patients and their families for participating in this study.

Authors' contributions

Wen-Xu Yang performed the investigations related to this study, drafted the manuscript, and interpreted the data. Lin Li, Hai-Rong Wu, and Song-Tao Wang helped with the data acquisition and analysis. Hong Pan and Yu Qi helped with the revision of the manuscript.

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