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# The Wolf-Hirschhorn Syndrome in Fetal Autopsy – A Case Report –

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Wolf-Hirschhorn syndrome (WHS) is a malformation associated with a hemizygous deletion of the distal short arm of chromosome 4. Herein we report a fetal autopsy case of WHS. A male fetus was therapeutically aborted at $17^{+0}$ weeks gestational age, due to complex anomaly and intrauterine growth retardation, which were found in prenatal ultrasonography. His birth weight was 65 g. Mild craniofacial dysmorphism, club feet, bilateral renal hypoplasia, edematous neck, and left diaphragmatic hernia of Bochdalek were found on gross examination. On GTG-banding, the fetus revealed 46,XY,add(4p) karyotype and the mother revealed 46,XX,t(4;18)(p16;q21.1), with normal karyotype of the father. Array comparative genomic hybridization performed on the autopsied lung tissue revealed loss of 4p16.2→4pter and gain of 18q21.1→18qter, suggesting 46,XY,der(4)t(4;18)(p16.2;q21.1)mat of fetal karyotype. This suggested deletion of 4p, compatible with WHS inherited from the mal-segregation of a maternal translocation t(4;18)(p16.2;21.1). Therefore, our fetus was both genotypically and phenotypically compatible with WHS.

**Key Words:** Autopsy; Karyotyping; Comparative genomic hybridization; Wolf-Hirschhorn syndrome

Wolf-Hirschhorn syndrome (WHS) is one of multiple congenital anomaly/mental retardation (MCA/MR) syndromes and is caused by partial deletion of the short arm of chromosome 4, particularly in the Wolf-Hirschhorn syndrome critical region-1 (WHSCR1) and Wolf-Hirschhorn syndrome critical region-2 (WHSCR2), which are located in chromosome 4p16.3. Loss of these regions is indeed the cause of WHS, and the length of deletion regions was associated with the specific clinical phenotype.[1] In many cases, WHS is caused by *de novo* deletion of the short arm of chromosome 4, including 4p16.3. A familial translocation was found in about 5-13% of WHS cases.[2] The incidence of WHS was estimated from about 1 in 20,000 to 1 in 100,000.[1,3] The key features of the WHS are, as follows: typical "Greek helmet" face (i.e., hypertelorism and frontal bossing, with prominent bridge of nose), microcephaly, cleft palate, severe psychomotor and growth retardation, low birth weight, diminished fetal activity, hypotonia, seizures and renal anomalies, which express various phenotypic features. Rarely, diaphragmatic hernia has also been reported.[4] This report pertains to an autopsy case of WHS, of which chromosomal studies were verified by array comparative genomic hybridization (array-CGH).

## CASE REPORT

The proband was the second male fetus of unrelated healthy parents, with no history of teratogenic exposures, such as drugs or gestational diabetes mellitus. The mother took iron supplements from the first trimester. No peculiarity was noted during the first trimester. At 16 weeks gestational age, prenatal ultrasonography showed the undersized male fetus in relation to gestational age, with congenital diaphragmatic hernia and thickness of the nuchal skinfold. The amniotic fluid index was within normal range.

The fetus was therapeutically aborted at $17^{+0}$ weeks gestational age (Fig. 1). The fetus was small for gestational age (birth weight, 65 g; femur length, 3 cm; abdominal circumference, 3 cm). The gross examination showed mild craniofacial dysmorphism, including mild frontal bossing and hypertelorism, club feet, bilateral small kidneys, edematous neck and congenital diaphragmat-

ic hernia of Bochdalek type. Microscopic examination showed a low number of nephrons and slightly hypertrophic glomeruli, with microlithiasis in the left kidney, immature lungs and dilated subcutaneous lymphatics on the neck, suggesting nuchal hygroma.

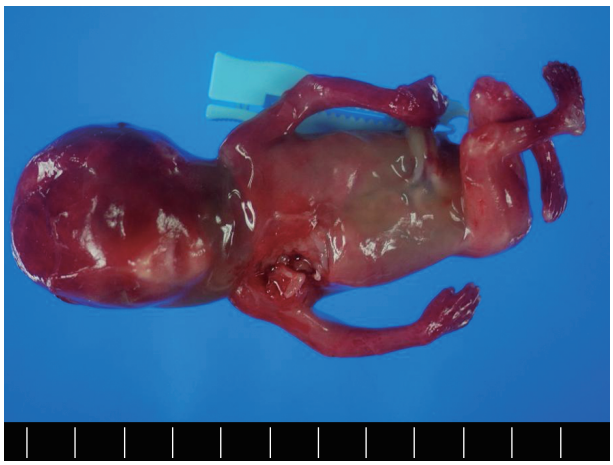

**Fig. 1.** The fetus shows mild craniofacial dysmorphism, i.e., the 'Greek helmet' anomaly, including mild frontal bossing and orbital telorism, thick nuchal skin-fold and club foot.

Chromosome analyses were carried out by GTG-banding at a resolution of 550 bp, using fetal tissue and parents' peripheral blood lymphocytes. The fetus revealed 46,XY,add(4p) karyotype (Fig. 2A) and the mother revealed 46,XX,t(4;18)(p16;q21.1) (Fig. 2B).[5] The father's karyotype was normal.

Because we did not have fresh frozen tissue, we extracted DNA from formalin-fixed and paraffin-embedded (FFPE) lung tissue. FFPE tissue is not always suitable for molecular analysis, because of formalin-induced DNA degradation. Nevertheless, Linn *et al*.[6] successfully applied 2 to 4 μg of FFPE DNA to array-CGH and reported similar results to those obtained from fresh DNA. In the case reported herein, DNA extraction and purification were carried out by standard protocols. The 3 μg of DNA was used for array-CGH analysis. Array-CGH was performed using the MacArray™ Karyo 4500 kit (Macrogen, Seoul, Korea), according to the manufacturer's protocol. We defined chromosomal

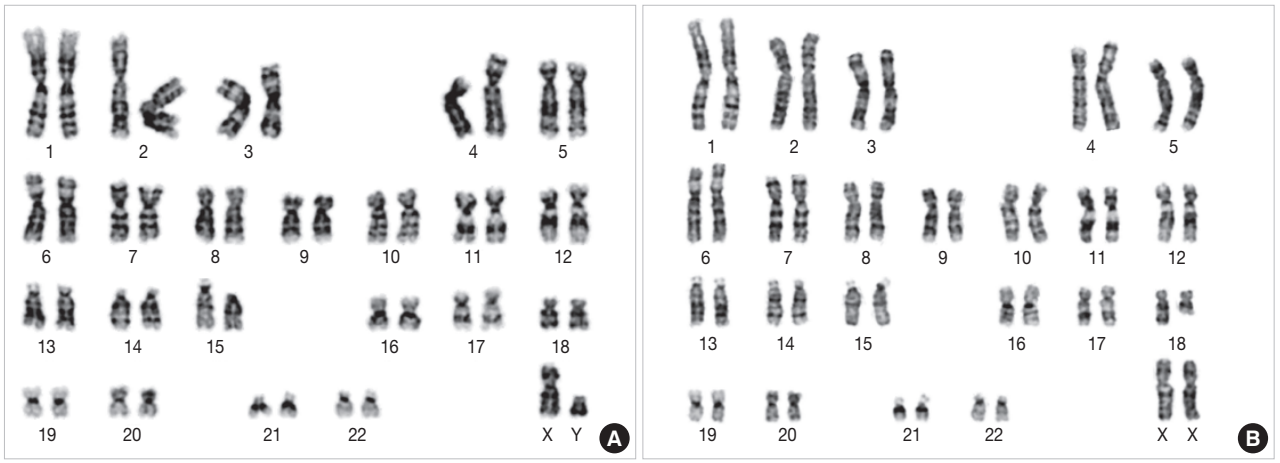


**Fig. 2.** Cytogenetic findings with GTG-banding (resolution: 550 bands). (A) Fetus: 46,XY,add(4p). (B) Mother: 46,XX,t(4;18)(p16;q21.1).

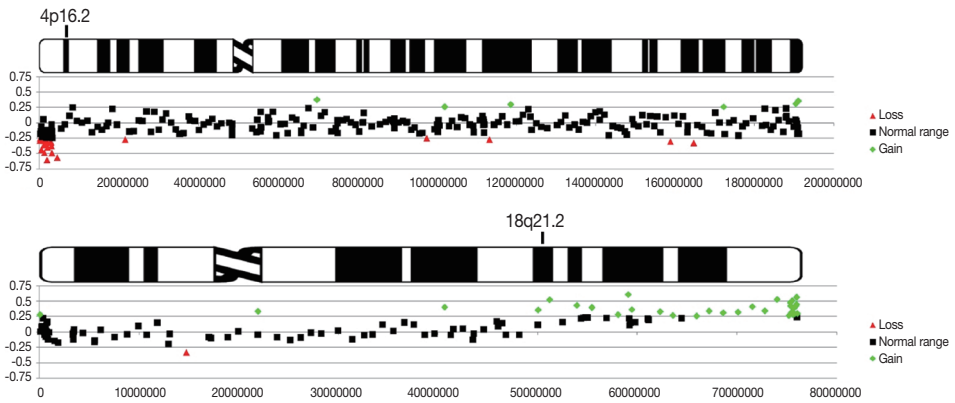


**Fig. 3.** Comparative results of the array genomic hybridization and idiogram. Red spot: loss below -0.25 normalized $\log_2$-ratio, green spot: gain above 0.25 normalized $\log_2$-ratio, black spot: between -0.25 and 0.25 normalized $\log_2$-ratio. Thus, the chromosome 4p showed chromosomal loss and chromosome 18q showed chromosomal gain (above: chromosome 4, below: chromosome 18).

gain as above 0.25 normalized $log_2$-ratio and chromosomal loss as below -0.25 normalized $log_2$-ratio. In our case, the result of array-CGH revealed 119 chromosomal gains and 114 chromosomal losses in 4,362 investigated human bacterial artificial chromosome (BAC) clones. In particular, it showed loss of 4p16.2→4pter and gain of 18q21.1→18qter (Fig. 3). When we correlated the GTG-banding karyotype of the fetus and mother with the result of the array-CGH, the fetal karyotype was finally designated as 46,XY,der(4)t(4;18)(p16.2;q21.2).arr 4pterp16.2 (76,938→ 4,559,231)x1, 18q21.2qter(50,007,394→76,110,688) x3 mat (pter means terminal part of p arm, qter means terminal part of the q arm, arr means the result obtained from microarray, and mat means from the mother).[5] Therefore, the fetal karyotype was actually loss of 4p and gain of 18q, which strongly supported the diagnosis of WHS.[7]

## DISCUSSION

In the GTG-banding study, the fetal karyotype showed chromosome 4p addition and normal chromosome 18. The main differential diagnosis was trisomy 4p. Trisomy 4p syndrome is due either to mal-segregation of a parental balanced translocation, or to *de novo* duplication.[8] However, the phenotypic and cytogenetic features of the fetus presented here were not indicative for the diagnosis of trisomy 4p syndrome. Combining the chromosomal study of both fetus and mother and the multiple anomalies found on autopsy, we hypothesized that chromosome 4p addition was composed of del(4)(p16.2→pter) and add(18)(q21.1→qter)mat. From the syndromes usually related to 4p addition and/or duplication 18q syndrome, such as WHS, Pitt-Rogers-Danks syndrome (PRDS)[9] and Lambotte syndrome,[10] our case mostly fit the WHS (Table 1). This hypothesis was supported by the following clues:

1) The mother's karyotype was 46,XX,t(4;18)(p16;q21.1).
2) Renal hypoplasia and club feet, although not specific, are also common in WHS.
3) Congenital diaphragmatic hernia, although rare in WHS, is one of the components of WHS[11,12] and it has not been reported in PRDS.[13]
4) Cystic hygroma, growth retardation, and mild craniofacial dysmorphism can also be clues for the diagnosis of WHS.[14]

**Table 1.** Commonly observed abnormalities in the Wolf-Hirschhorn syndrome and other syndromes

| Major features | Common features in WHS[3,4] | Trisomy 4p syndrome[8] | Duplication 18q syndrome[4] | Our fetus |
|---|---|---|---|---|
| Head and neck | Frontal bossing<br>High frontal hairline<br>Hemangioma<br>Stenosis or atresia of nasolacrimal ducts<br>Small mandible<br>Scalp defect | Microcephaly<br>Ocular abnormalities<br>Short neck | Maxillary hypoplasia<br>Micrognathia | Mild frontal bossing<br>Mild orbital telorism<br>Nuchal hygroma |
| Ears | Simple, posteriorly angulated or low-set ears | "Low-set" or posteriorly angulated | Hypoplastic ears | Hypoplastic lobule of left ear |
| Eyes | Extropia and ectopic pupils<br>Proptosis<br>Hypertelorism<br>Up-slanting palpebral fissures<br>Ptosis | Ocular abnormalities<br>Hypertelorism | Congenital cataracts<br>Short palpebral fissures<br>Upper epicanthal folds | Hypertelorism |
| Nose | Short prominent philtrum<br>Broad and beaked nose<br>Prominent bridge of nose and shallow septum | Depressed or flat nasal bridge, bulbous tip | | Depressed nose |
| Mouth and oral structures | Down-turned corners of mouth | High arced palate | Carp-shaped mouth<br>Mal-eruption, malocclusion of the teeth | NA |
| Chest | | Chest abnormalities | Congenital heart defect | |
| Hand and foot | Abnormalities (non-specific) | Abnormalities (non-specific) | Camptodactyly<br>Rocker-bottom feet<br>Crowing of toe | Club feet |
| Urogenital system | Hypoplastic kidney | Abnormal genitalia in male | Pseudohermaphroditism ectopic kidneys | Hypoplastic kidney |

WHS, Wolf-Hirschhorn syndrome; NA, not available.

5) Sporadic case in this family.[10]

However, the fetus did not show the typical "Greek helmet" face and mental retardation could not be assessed because of too young fetal age (i.e., 17 weeks gestational age). Faced with limited clinical data, we carried out the additional cytogenetic study to reach an accurate diagnosis of WHS. Cytogenetic abnormalities are a major cause of MCA/MR syndromes. In many cases, the cytogenetic study on prenatal diagnosis and screening is conducted by conventional microscopic analysis, using GTG-banding. However, the conventional microscopic cytogenetic study is sometimes unable to detect either microdeletion or microduplication, whereas array-CGH has demonstrated a highly diagnostic yield in MCA/MR syndrome patients, including WHS.[15,16] In addition to the GTG-band karyotype study, molecular studies can be used to identify deletion of the WHSCR1 and/or WHSCR2 region on 4p. In many cases, both metaphase fluorescence *in situ* hybridization and array-CGH have been recently used to check for chromosomal anomaly.[17-19] Both methods can be useful for identification of 4p16.3 deletion. Nevertheless, the array-CGH study is more informative to determine the length of either deletion or duplication associated with the clinical phenotype. The array-CGH was initially developed as a research tool for investigation of the genomic imbalances in cancer, and it is currently used to detect aneuploidies, well-characterized microdeletion/microduplication syndromes and subtelomeric or other unbalanced chromosomal rearrangements. Therefore, it is now considered as a useful tool for prenatal diagnosis.[15,20] Moreover, recently DNA extraction and purification are routinely performed in many laboratories and surgeons are more familiar with them and other methods. The microarray CGH delivered the accurate karyotype of our case and made the diagnosis of WHS.

During the autopsy, it is sometimes difficult to confirm the diagnosis because fresh tissues are not routinely kept at deep freezing temperature. However, with appropriate DNA extraction from the FFPE tissue, array-CGH can be a useful additional tool to identify genetic diseases and provide genetic information in fetal and pediatric autopsy cases.

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