**Comparative Genomic Hybridization (CGH) for Prenatal Diagnosis of Wolf-Hirschhorn Syndrome**

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**Abstract**

Wolf-Hirschhorn Syndrome (WHS) is a rare genetic condition caused by partial deletion of the short arm of chromosome 4 (4p-). Variability in 4p deletions and rearrangements leads to a wide spectrum of clinical manifestations of this disease. Most prenatal WHS diagnoses are associated with large 4p deletions identified by conventional cytogenetic techniques; however some submicroscopic deletions can only be diagnosed using molecular techniques. In this case report, a combined diagnostic approach based on conventional karyotyping and comparative genomic hybridization (CGH) offered a quick and definitive result to allow accurate diagnoses and genetic counseling of WHS for the family.

**Key words**

Wolf-hirschhorn syndrome; Array-comparative genomic hybridization (array-CGH); Prenatal diagnosis; 4p16.3 deletion; "Greek warrior" helmet profile.

**Introduction**

Wolf-Hirschhorn Syndrome (WHS), also known as deletion 4p or 4p- syndrome, is a well known clinical condition caused by deletions of variable amplitude in the chromosomal region 4p16.3 [1]. WHS was first described in 1965 in two independent publications by Wolf et al. [2] and Hirschhorn et al. [3]. The prevalence of this syndrome varies between 1:20,000 and 1:50,000 births, with a 2:1 bias in favor of females [4].

Factors for the great phenotypic variability that characterizes WHS include extent of the 4p deletion, the complexity of the basic genomic defect and the severity of seizures. However, the main clinical features include: pre and postnatal growth delay, profound psychomotor retardation, seizures, skeletal abnormalities, craniofacial dysgenesis (microcephaly, prominent glabella, widely spaced and prominent eyes, a "Greek warrior helmet appearance" of the nose, hypertelorism, cleft lip/palate, plump lips), heart defects and urinary tract malformations [5-9].

Most prenatally diagnosed cases of WHS are associated with large 4p deletions identified by conventional karyotype. However, with the widespread use of new molecular techniques such as array comparative genomic hybridization (a-CGH), the diagnosis of submicroscopic chromosomal aberrations associated with WHS critical regions could be improved, as is currently the case for many other complex genetic syndromes [5].

**Clinical Report**

The patient was a 31-year-old healthy primigravida with no medical or surgical history, no Diabetes Mellitus, no toxic habits, no malformations or genetic syndromes in her family or that of her husband, and non-consanguineous parents. The patient received regular treatments with multivitamins including potassium iodine and folic acid. The first trimester ultrasound, performed with an abdominal probe (Voluson ProV), showed a live fetus with crown-rump length of 66.7mm, equivalent to 13 weeks and 2 days gestation.

**Figure 1:** In the 20th week, the ultrasound evaluation show a hypoplastic nasal bone, measuring 1.9mm (abdominal probe, Toshiba Xsario X6).
and a nuchal translucency of 1.5 mm. Additional chromosomal markers were normal (nasal bone present and positive a-wave flow in the Ductus Venosus), except for the presence of tricuspid regurgitation with normal cardiac morphology. No other structural malformations were observed.

Biochemical markers in the first trimester, analyzed by Elecsys analyzer (Roche®), were in the normal range (B-hCG: 0.319 MoMs, PAPP-A: 1.828 MoMs). The risk for chromosomal abnormalities was calculated by using the FMF® module of the Astraia computer system, and was reported to be 1:3.833 for trisomy 21 and 1:20.000 for trisomy 13 and 18.

In the 20th week, a routine ultrasound, performed with an abdominal probe and ultrasound machine Toshiba Xsario X6, revealed a live fetus with biometry according to symmetric growth restriction (3rd centile for the gestational age) and estimated fetal weight, by Hadlock algorythm, of 201 grams. The ultrasound evaluation also showed a hypoplastic nasal bone (1.9mm) (Figure 2) without nuchal edema collapsed stomach and a single umbilical artery (Figure 2). Tricuspid regurgitation persisted and the fetal heart ultrasound was once again normal (Figure 3). Because of the risk of a genetic condition in the fetus, an amniotic fluid study was offered to the patient, and amniocentesis was carried out.

qF-PCR, conventional karyotyping and array-CGH were requested. 7 days after the procedure the results of qF-PCR reported normal 21, 13, 18 and sex chromosomes. 14 days after the procedure, array-CGH indicated a pathogenic duplication in the 3p26.3p24.3 cytoband of 19,7 Megabases (Mb) and 78 genes OMIM, as well as pathogenic deletion in the 4p16.3p16.1 cytoband of 9.5 Mb and 67 genes OMIM, affecting the entire WHS critical region and suggesting an unbalanced rearrangement between chromosomes 3 and 4 [t (3, 4) (p24.3, p16, 1)] Figure 4. A month after the amniocentesis, karyotype information showed an addition on the short arm of chromosome 4 with an atypical pattern of bands that could not be determined (Figure 5). Based on these results, prenatal diagnosis of WHS was made and the patient decided to terminate pregnancy at 24 weeks gestation. A female fetus weighing 300 grams was obtained.
translocation, not more than 10-14%. The majority, about 85-90%, mostly due to balanced rearrangements.

In prenatal diagnosis of WHS, the main sonographic finding is a severe and early intrauterine growth restriction (percentile<3) which may be associated with multiple congenital malformations such as craniofacial abnormalities (microcephaly, hypertelorism, prominent glabella, high forehead and low-set ears), midline defects like corpus callosum agenesis, cleft lip and palate, and atrial or ventricular septal defects [7,10]. Other malformations such as cystic hygroma, ventricular cysts, pulmonary hypoplasia, gall-bladder agenesis, congenital diaphragmatic hernia and club hand and clubfoot have also been reported prenatally. Their frequency varies from 10% to 20% [11].

Several studies have underlined the importance of a strong genotype-phenotype correlation in WHS [8,12]. Thus, three different phenotypes are usually described: Micro deletions less than 3.5 Mb in size are associated with a mild phenotype represented by a typical facial appearance without microcephaly and growth retardation. Deletions between 5 and 18 Mb are associated with the classical WHS phenotype, represented by a severe psychomotor retardation. Deletions between 5 and 18 Mb are associated with the classical WHS phenotype, represented by a severe psychomotor retardation. Deletions larger than 22 Mb, a severe phenotype with major growth retardation, septal defects and minor deletions [7,10].

In conclusion, growth restriction as an isolated finding or associated with facial dysmorphism and other major malformations may be suggestive of WHS, and should trigger genetic investigation. A combined diagnostic approach based on conventional karyotyping and molecular analysis can offer a quick and definitive result to allow accurate diagnoses. Parents must be studied by conventional cytogenetics and by FISH to search for a parental balanced translocation that allow genetic counseling for the family.

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